Quality Assurance Project Plan for Targeted Fish Tumor Surveys for

Brown Bullhead (*Ameiurus nebulosus or Ictalurus nebulosus*) and/or

Other Fish Species

Beneficial Use Impairment Advancements
for the Massena/Akwesasne Area of Concern
on the St. Lawrence River.
GL-97221310-0

January 2012

Prepared for

U.S. Environmental Protection Agency Region 2 and
Great Lakes National Program Office

Prepared by

Jessica Jock, Project Manager
St. Regis Mohawk Tribe
Environment Division
Akwesasne, 13655

Quality Assurance Project Plan Title and Approval Sheet

Project title: **Fish Tumor Survey – Target Species Bullhead**
Massena/Akwesasne AOC St. Lawrence River - BUI Advancement

Prepared by: **SRMT Environment Division**

Approvals:

Jessica L. Jock, BUI Coordinator, SRMT Environment Division	Date
Barbara Belasco, Project Manager, USEPA Region 2	Date
Kai Tang, USEPA Region 2 Quality Assurance (QA) Officer, Tribal Liaison	Date
Donna Ringel, USEPA Region 2 Quality Assurance (QA) Manager	Date
Angela Benedict, SRMT Quality Assurance Officer (QAO)	Date

Project Initiation:	April1, 2012
Project Termination:	September30, 2013
Sampling Operations:	St. Regis Mohawk Tribe
Operations Manager:	Jessica L. Jock, SRMT Environment

Ken Jock, SRMT Environment Division Director's Approval	Date

Distribution List

Barbara Belasco
EPA Project Officer
U.S. EPA - Region 2
290 Broadway
New York, NY 10007
(212)-637-3848
belasco.barbara@epa.gov

Marcos Santos
U.S. EPA – Region 5
Grants & Contract Mgt Branch
77 West Jackson Blvd
Chicago, Illinois 60604
(312)-353-3490
Santos.marco@epa.gov

Ken Jock
St. Regis Mohawk Tribe
Environment Division Director
412 State Route 37
Akwesasne, 13655
(518) – 358-5937
ken.jock@srmt-nsn.gov

Rodger Klindt
NYSDEC, Region 6
Aquatic Biologist
317 Washington St.
Watertown, NY 13601
(315)-785-2263
rmklindt@gw.dec.state.ny.us

Angela Benedict
SRMT Environment Division
Tribal QA Officer
Akwesasne, 13665
(518)-358-5937
(518)-358-6252 (fax)
angela.benedict@srmt-nsn.gov

Jay Wilkins
SRMT Environment Division
Wildlife Technician
Akwesasne, 13655
(518)-358-5937
(518)-358-6252 (fax)
jay.wilkins@srmt-nsn.gov

Donna Ringel
U.S. EPA -Region 2
QA Program
Division of Environmental Science and Assessment
2890 Wood Bridge Ave.
Edison, NJ 08837
(732)-321-6760
Ringel.Donna@epa.gov

James Snyder
SRMT Environment Division
Fish and Water Technician
412 State Route 37
Akwesasne, 13655
(518) – 358-5937
(518)-358-6252 (fax)
james.snyder@srmt-nsn.gov

Ann Casey
Technical Service Program Manager
PACE Analytical Services, Inc
2190 Technology Drive
Schenectady, NY 12308
(518)-346-4592
Ann.Casey@pacelabs.com

Paul R. Bowser, PhD
Aquatic Animal Health Program
College of Veterinary Medicine
Cornell University
Ithaca, NY 14853-6401
(607) – 253-3384
prb4@cornell.edu

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Section 1.0 PROJECT/TASK ORGANIZATION

This Quality Assurance Project Plan (QAPP) describes the Quality Assurance (QA) and Quality Control (QC) activities and procedures associated with field collection of targeted species (bullhead or suckers) to conduct external and internal anomaly and liver lesion analysis. Project tasks will be carried out by key personnel and methodology listed below.

1.1 Project Management

The Project will be implemented by the St. Regis Mohawk Tribe's Environment Division. Within our Division, we have the technical and sampling equipment support to conduct the study. Some portions of the field collection will be assisted by Fisheries Biologist from NYSDEC Watertown, Region 6 office. This section includes an organization chart showing lines of authority and reporting responsibilities (Figure 1).

EPA Project Manager
Barbara Belasco will be responsible for providing funding and QAPP approval, and coordination with the proposed project and reporting needs.

SRMT Environment Director
Ken Jock, SRMT Environment Director, is responsible for providing financial and staff resources necessary to meet project objectives and implement the requirements described in this QAPP. The Director is the official project contact of all funding resources and has primary signing authority for all purchases, project plans and correspondences.

Quality Assurance (QA) Officers
Angela Benedict, Tribal QA Officer is responsible for reviewing and approving all QAPPs according to the Tribe's Quality Assurance Management Plan, 2007 (QAMP) and Quality Assurance Management Project Plan (QAPP) Guidance, September 6, 2001. She has signing authority for a project work plan for the SRMT Environment Division. Angela is also responsible for conducting Audits and Assessments of work done throughout the sampling program, and coordinating QAPP approval with Region 2 QA Manager (Donna Ringel) and Tribal QA Liaison (Kai Tang). Should there be a need for any corrective actions, Donna and/or Kai will be contacted and consulted with prior to any continued work.

SRMT Project Manger
Jessica Jock, Environmental Specialist and Beneficial Use Impairment (BUI) Coordinator, will be responsible for implementing study design, directing contractors, ensuring adherence to study design and accomplishment of project objectives, coordinating with other office departments involved with the sampling, sampling oversight, reporting quarterly to EPA, and final report compilation.

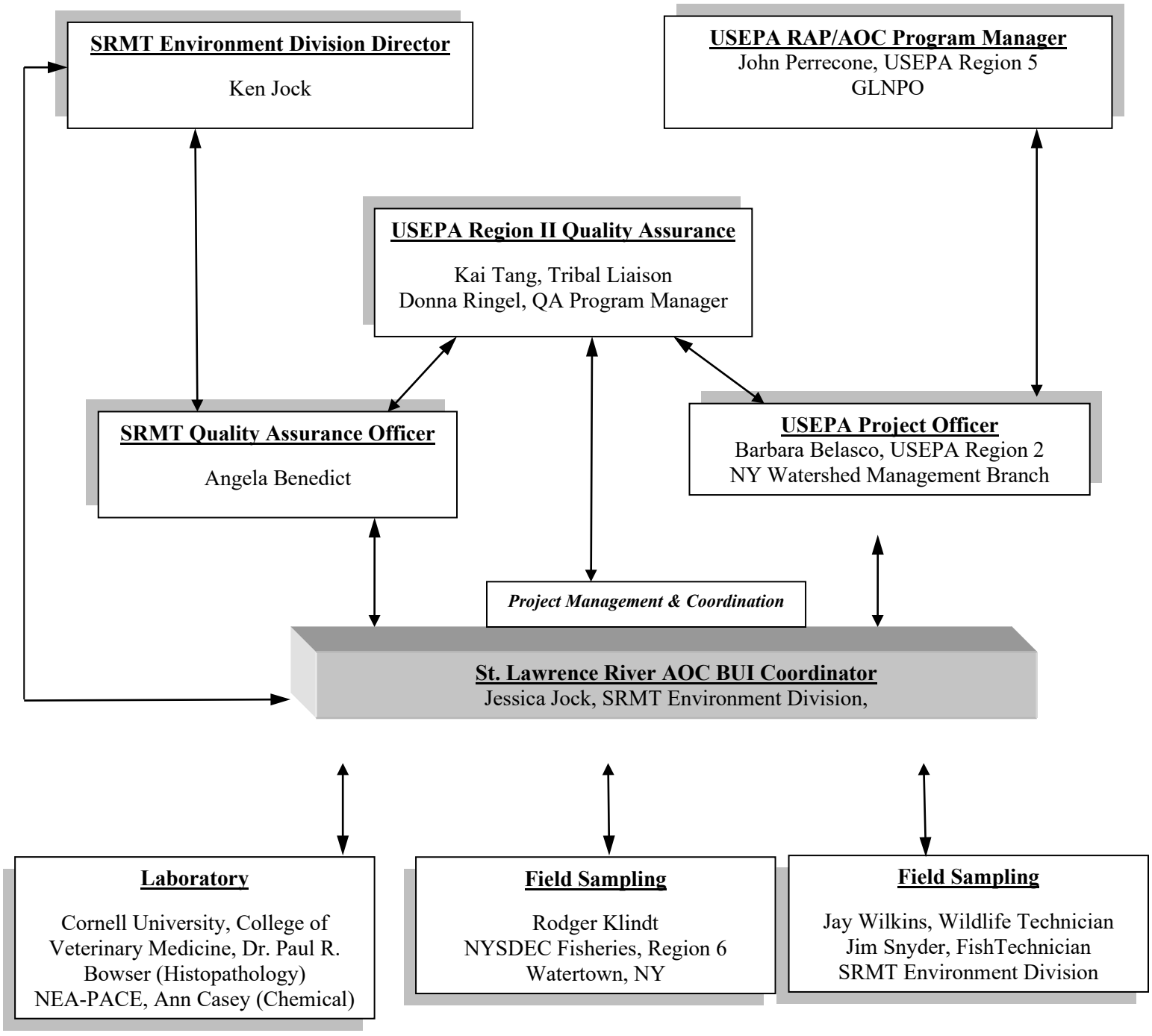
Field Crew
Jay Wilkins, Wildlife Technician, will act as Field Crew Leader in the field for all collection and fish processing activities. SRMT Fishery Technician Jim Snyder will command the boat navigation responsibilities and assist with the field collection and processing. New York State DEC Fishery Biologist, Rodger Klindt will operate the electro fishing boat, and assist with initial processing efforts,

and aging techniques, when available. Field tasks include deployment and monitoring, maintenance and repairs of sampling equipment, field collection, observation, and tissue extraction. SRMT staff will also be responsible for documentation and records to be maintained in the field. They will be responsible for all field data supply check lists (Appendix A & B), Field Data Sheets (Appendix C)labeling, recording, management, and mapping GPS locations of sampling sites. They are expected to follow the methodologies outlined in this QAPP.

Laboratory– Histopathology
Fish will be collected following procedures outlined in *Field Manual for Assessing Internal and External Anomalies in Brown Bullhead (Ameiurus nebulosus)* attached as Appendix C, and complemented by instructions, tables & figures, Fish Health Data Sheets for internal organs, and diagrams included in *Illustrated Field Guide for Assessing External and Internal Anomalies in Fish* (Appendix D). Fish livers will be submitted to Dr. Paul R. Bowser, Cornell University, College of Veterinary Medicine. The laboratory will follow procedures outlined in *Manual for the Microscopic Diagnosis of Proliferative Liver and Skin Lesions in the Brown Bullhead (Ameiurus nebulosus)* attached as Appendix E. There is no certification for the fish pathology laboratory. Dr. Paul R. Bowser will be assigned the Fish Pathologist Project Manager that will be responsible for reviewing QAPP to verify histopathology operations will meet project requirements, reviewing receipt of shipped samples and chain of custody, reporting on discrepancies within 24 hours of receipt, notifying SRMT project manager regarding nonconformance or problems and implementing corrective actions.

Laboratory– Chemical Analysis
Fish will be collected following procedures outlined in *Field Manual for Assessing Internal and External Anomalies in Brown Bullhead (Ameiurus nebulosus)* attached as Appendix C. Fish fillets of every 10^{th} fish (20% of sample size) will be submitted to NEA – A Division of Pace Analytical Services (NEA-PACE)laboratory for PCB, PAH, and % lipid analysis. NEA-PACE is a certified Environmental Laboratory Accreditation Program (ELAP) for environmental analysis. Laboratory accreditation is outlined in Appendix F and SOPs for analytical procedures are outlined in Appendix G. The lab assigned Project Manager Ann Casey will be responsible for reviewing QAPP to verify that analytical operations will meet project requirements, reviewing receipt of shipped samples and chain of custody, reporting on discrepancies within 24 hours of receipt, notifying SRMT project manager regarding nonconformance or problems and implementing corrective actions.

Figure 1. Organization Chart of Management Fish Tumor Study



Section 2.0 PROBLEM DEFINITION/BACKGROUND

This section describes the problem addressed by this project funding, and provides background and historical information that supports the need for the project.

2.1 Problem Definition

Identified as one of five Bi-National Areas of Concern (AOC) by the International Joint Commission (IJC) under the Great Lakes Water Quality Act (GLWQA), the proposed project will take place in the St. Lawrence River AOC Watershed, primarily in St. Lawrence County and the territory of Akwesasne. The Massena/Akwesasne AOC includes the Grasse (04150304), Raquette (04150305), and St. Regis (04150306) tributaries, Power Canal on the Grasse River, and portions of the St. Lawrence River.

Proposal efforts include collecting the needed information to make progress on the Fish Tumors or Other Deformities Beneficial Use Impairment (BUI), targeting brown bullhead, the approved indicator species by the IJC. Delisting Criteria established for the St. Lawrence River AOC include documentation that incidence rates of fish tumors and reproductive deformities do not exceed those observed at control sites, and survey data confirms the absence of neoplastic or preneoplastic liver tumors (Table 1). The current status of this BUI is "Unknown/Needs Further Assessment", with no internal surveys conducted to date site specific to the Massena AOC.

The Massena/Akwesasne Area of Concern (AOC) has previously been identified as an AOC due to discharge of hazardous industrial byproducts to nearby tributaries, land, and the St. Lawrence River itself, thus causing ecological impairments and impaired water quality. Three industrial facilities in Massena, NY were known to have historically released discharges that contained persistent organochlorine chemicals (POCs) such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), dibenzo-p-dioxins, dibenzofurans, dichlorodiphenyldichloroethylene (DDE), mirex, as well as nutrients and metals into the Massena Area of Concern.

PCBs have been listed as the primary contaminant of concern for all three of these sites (General Motors, Reynolds Metal (now Alcoa East) and Aluminum Company of America (now Alcoa west), but cleanup threshold for PAHs were developed for risk reduction goals as well. PAHs are assumed to be associated with the St. Lawrence River Area of Concern (AOC) due to the aluminum processing plants. "One of the most important industrial sources of PAH is the production of Al by electrolysis using the Soderberg anode. The electrode used in the process is a paste consisting of coal tar and pitch (Thrane, 1986)." The Soderberg potline is still used today at Alcoa East, while Alcoa West has changed to Carbon Anodes processes. Of particular interest for this QAPP, PAHs have been shown to be associated with skin and liver tumors of bullhead at other sites in the Great Lakes.

Brown bullheads are commonly affected with epidermal neoplasm of the mouth and skin, where neoplasm is defined as an abnormal tissue that grows by cellular proliferation to form a distinct mass of tissue. The neoplasm are diagnosed as either papillomas (benign) or carcinomas (malignant) and occur singly or in multiples varying in size from several millimeters to several centimeters. Bullhead are a species of choice due to their abundance, bottom-feeding and sediment burrowing behaviors, tolerance, human consumption concerns for bioaccumulation, and prevalence across the Great Lakes nearshores.

The overall objective of this survey is to evaluate the status of Fish Tumor and Other Deformities beneficial use impairment and to make progress in understanding the impairment specific to the Massena AOC. This study will determine tumor prevalence and examine possible association between tumor prevalence and contaminant exposure to PCBs and PAHs. It is hypothesized that incidence rates of

internal and external fish tumors within the Massena/Akwesasne St Lawrence River Area of Concern will not exceed an acceptable incident rate to comparable sites outside the Area of Concern.

Specific Objectives include:

- Assess the prevalence of tumors or other deformities in AOC fish; and
- Prevalence and severity of external tumors and other deformities in bullheads from the AOC compared with background (as per Appendix C); and
- Prevalence and severity of liver tumors in bullheads from the AOC compared with background (as per Appendix E); and measure
- Concentrations of PCBs and PAHs in 20% of the bullhead sent for liver histopathology confirmation targeted from the AOC compared with 20% of the bullhead targeted from background*. If the PCBs and PAH concentrations are above background, then the concentrations will be compared to appropriate literature values and other related scientific studies to determine the potential significance of the concentration levels; and
- Evaluate and weigh the gonads, liver, and spleen for index measurements for health and reproduction observations (as per Appendix D, Internal Examination Field Sheet, pg. 42).

*"Background" refers to "Outside the AOC" sampling locations. It will be a comparison of years 2012 and 2013 "Inside AOC" to "Outside AOC" (i.e. background). If fish tissue concentrations in the AOC are significantly different than background concentrations in 2012 and 2013, then a review of published data on adverse effects associated with abnormalities and reproduction as well as other secondary data will be reviewed. It is already anticipated that fish tissue concentrations in the AOC are above outside AOC or background. Use of Secondary Data has been outlined in Section 15.0.

Table 1. St. Lawrence River, Massena/Akwesasne AOC Beneficial Use Impairments, Status and Delisting Criteria for Fish Tumors or Other Deformities (Ecology and Environment, 2008).

Fish Tumors or Other Deformities	Unknown/Needs Further Assessment	Incidence rates of fish tumors or other deformities do not exceed rates at unimpacted control sites; AND Survey data confirm the absence of neoplastic or preneoplastic liver tumors in bullheads or suckers as compared to control sites; AND No reproductive deformities in observed resident species as compared to control sites

2.2 Background

Several remedial efforts have occurred to remove contaminant sources, improve water and sediment quality in and around the AOC between 1995-2009. Although many chemicals exist in the AOC, the primary contaminant of concern identified at all three industrial sites for remedial action by USEPA has been PCBs, but phenols and VOCs (General Motors, 1995), PAHs and dibenzofurans (former Reynolds Metal, 2001 & 2009) have also been listed for remedial action. In SRMT's database of historical surface sediment total PAH concentrations (mg/kg), the St. Lawrence River around former Reynolds Metal had the highest detection of total PAHs at 3,804 ppm. Data points ranged from non-detect to 3,804ppm, a mean value of 129 ppm, and a median value of 10ppm for the St. Lawrence River near the industry. The Grasse River had the second highest total PAH concentrations with a range of non-detect to 690ppm, a mean value of 79ppm and a median value of 15ppm. The Power Canal and Raquette River had lower detections of total PAHs, 0.12-27.0ppm and <2.62-14.08 ppm respectively. The St. Regis River did not have an applicable dataset. The author assumes there was no targeted sediment sampling for PAHs in the St. Regis River since there is no direct source from either of the smeltering plants (Alcoa and former Reynolds Metal). Data points were confirmed to be representative of the data available on-line through NOAA's St. Lawrence River Watershed Database and Mapping Project Software and Data (http://response.restoration.noaa.gove/index.php)

The St. Lawrence River Remediation Project (SLRRP) was a removal action conducted adjacent to the former Reynolds Metals Company in 2001. This removal effort included targeting sediments containing total PAHs greater than 10mg/kg (ppm). Due to the onset of winter, mischaracterization of collocation of PCB and PAH contaminants, and dredging limitations, some PAHs remained after the 2001 removal effort. Subsequent sediment characterization specific to PAHs was conducted between 2002-2006, demonstrating PAH concentrations in the sediment in some locations >500 ppm total PAHs. Final remedial action was conducted in 2009 at the SLRRP, and dredge cells in excedance of 20 mg/kg (ppm) total PAHs were capped with an armor layer (USEPA, December 2008). The Grasse River remains a source of contaminated sediment in the AOC that is still in the Remedial Investigation and Feasible Study (RI/FS) Phase. It is expected that USEPA will select an appropriate remedy to mitigate risks to the biota, fish, human and ecological receptors by the end of this grant funding period (2013).

There can be multiple initiators and promoters of neoplasia. Tumors and deformities affecting the brown bullhead are hypothesized to be the results of viruses, parasitic invasion, hybridization, and contaminated sediment. Fish Pathologists will review the samples submitted for liver histopathology (see App. D and E). However, these referenced procedures will not differentiate amongst the possible causes. There have been sediment samples that show presence of PAHs and PCBs in sediment samples within the Massena/Akwesasne AOC. PAHs have been associated with causing liver tumors. Brown bullhead has been selected as the indicator species by IJC due to their burrowing and habitat selection in the sediment, thus increasing their external exposure to contaminants. Brown bullhead are targeted for this sampling effort, but in the event adequate sample size cannot be obtained, yellow bullhead (*Ameiurus natalis*) or white sucker (*Catostomus commersoni*) may also be collected. No sediment samples are proposed for this effort since it is not the objective of the Fish Tumor Delisting Criteria. The objective of the study is to document the incidence rates of fish tumors and reproductive deformities do not exceed those observed at control sites (background), and confirmation of presence/absence of neoplastic or preneoplastic liver tumors as outlined in Section 1.0 and Section 2.1.

The Brown Bullhead, (*Ameiurus nebulosus or Ictalurus nebulosus*) is a fish of the Ictalyridae family that is widely distributed in North America. It is a species of bullhead catfish and is similar to the Black Bullhead (*Ameiurus melas*) and the Yellow Bullhead (*Ameiurus natalis*). The brown bullhead thrives in a variety of habitats, including lakes, rivers, and ponds with low oxygen and/or muddy conditions. In many areas of the United States, Brown Bullheads are opportunistic bottom feeders. They eat insects, leeches, snails, fish, clams, and many plants. It is considered a warm water fish and inhabits both fresh and brackish waters. It is an extremely hardy fish. The brown bullhead is easily identified by its distinctive barbells, thick rounded body, large somewhat flattened head, scaleless dark brown skin, mottled sides, cream-colored belly, square caudal fin, and sharp, saw-toothed spines at the base of the dorsal and pectoral fins. Brown bullheads average 230 – 305 mm and rarely exceed 457 mm (Mugford 1969). A typical adult weighs approximately 454 g, but they may reach as much as 1,816 g (Scarola 1987). Brown bullheads are not territorial except during the breeding season, when the males defend the female and the nest (Adams and Hankinson 1926). Little is known about the home range of brown bullheads. Seasonal movement patterns and home ranges of brown bullhead have been studied within the Anacostia River, Washington DC. The linear home range averages were 0.5 km to 2.1km and remaining within 3.0 to 4.0 km of their release location. (Pinkney, 2005). Brown bullheads bury themselves in the soft mud bottom of lakes, ponds, and streams and remain inactive over the winter (Scarola 1987). This behavior typically occurs when water temperatures are between 0 – 18°C and is more common where lower temperatures are found (Loeb 1964, as cited in Carlander 1969). Brown bullheads spawn in the late spring when water temperatures reach 21°C. A shallow nest is excavated by one or both parents in mud or sandy substrate near the cover of logs, rocks, or vegetation, in water less than 2 ft deep. Bullheads lay 2,000 – 10,000 small (about 3.0 mm), cream-colored eggs in an adhesive cluster in the nest site. Both parents guard the eggs and aerate them by fanning, physically stirring them up, and taking them into the mouth and spitting them back out (Scarola 1987).

Section 3.0 PROJECT TASKS/DESCRIPTION

3.1 Measurements: Fish Analysis of Fish Tumors and Deformities

This project will determine presence/absence of liver neoplasm and incidence rates of external anomalies in fish within the Massena/Akwesasne St. Lawrence River Area of Concern (AOC). Studies Objectives will target appropriate habitat of target species to collect, observe external anomalies, and submit liver tissue for histopathology work, and a subset of fillet tissue for chemical analysis. Fillets are proposed for analysis because the question of concern is related to the body burden of contaminants to each individual fish. The chemical analysis will add another line of evidence that demonstrates what may bioaccumulate in the tissue that may be causing abnormalities (skin and liver) vs. the whole fish for an ecological perspective of bioaccumulation up the food chain to piscivorous consumers. Muscle tissue fillets (vs. liver) have been proposed to be consistent with Alcoa annual fish trend monitoring efforts, GM sampling in the St. Lawrence River and NYS Fish Procedures for Contaminant Analysis and Tissue Processing. Hoop net collection efforts will be utilized, and supplemented with electrofishing with the assistance of NYSDEC. This project is developed to provide data and assist a determination on the "Unknown/Needs Further Assessment" Status of the *Fish Tumors and Other Deformities* Beneficial Use Impairment (BUI), thus making progress on BUI Advancements for the Massena/Akwesasne AOC.

To be consistent with literature, sampling will be conducted during spring, early summer between the months of April-June. Sampling reach locations were selected based on several factors including:

- Accessibility
- Variability in habitat (water depths and presence of bullhead)
- Geographic location in the river
- Home range of bullhead species

The approach will involve collection 105 bullhead from within the AOC, and 105 bullhead outside the AOC for external and internal comparison for a total sample size of 210 fish collected per year. All 210 fish samples will be submitted for liver histopathology analysis, and a subset of 20% of fish for chemical analysis (inclusive of % lipids, PCBs, and PAHs). The reason why 20% of fish will be tested for chemical analysis it that, although the primary objective of the study is to document presence/absence of liver lesions/tumors and external abnormalities, chemical data will be another line of evidence to suggest if the presence of such abnormality findings (internal or external) may be associated with tissue concentrations. The 20% is what was budgeted during the 2010 GLRI proposal submittal.

The AOC and Outside AOC will represent the multiple tributaries included in the Massena/Akwesasne AOC (See Figure 2 and Figures 3-1:3-8). Fig. 2, Massena Area of Concern Study Area for Fish Tumors, will include the locations of any existing dams and CERCLA or State Listed Sites, in the Final Report. This process will ensure that fish data collected will be representative of a variety of habitats and locations throughout each river of interest, while remaining within the home range of the bullhead and meet sample size confidence intervals. Rafferty and Grazio Fish Tumor Manual suggest a sample size of 30-50 fish per site. Since there are four rivers of interest in the Massena AOC, our sample size will be on the lower end of acceptable sample size to allow for greater distribution throughout the AOC. Therefore, thirty-five (35) fish greater than 250 mm in length will be targeted at each Site, with a total of six (6) Sites sampled per year for a total of 210 fish/year. Equal sample size will be sampled Inside and Outside the AOC at appropriate river locations. Each fish will be collected for both external and internal analysis. Measurements for external anomaly scoring index, Heptosomatic index (HSI), Gonadosomatic index (GSI), length, age, and sex will be determined for each bullhead collected. If it is determined by the field

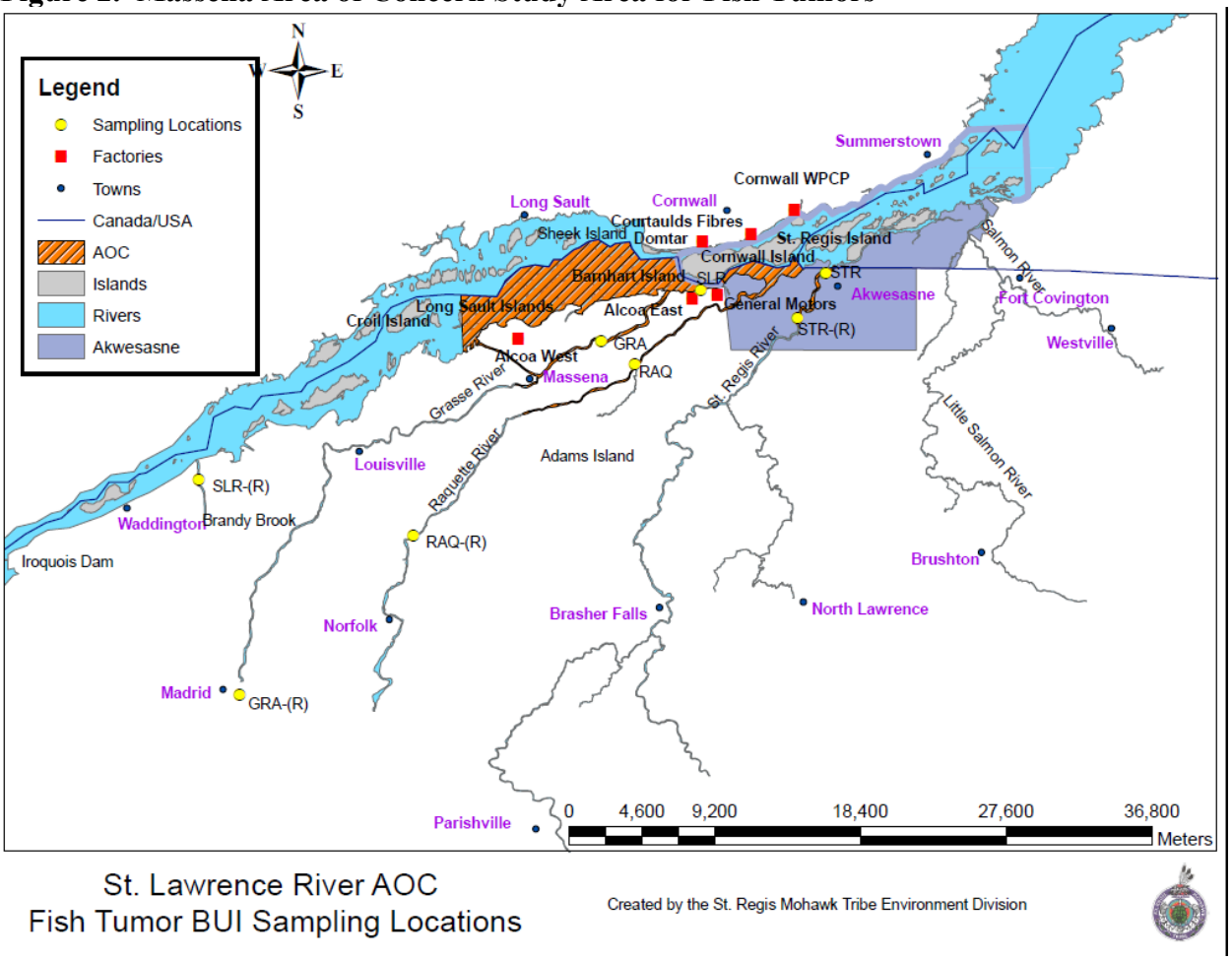
crew leader that the processing and measurement time for GSI is too intensive, it will be removed from the measurement collections. Time permitting, internal observations on the liver, bile, spleen, and gonads will be documented at the discretion of the field crew leader as per Section 6.3 in Appendix D and recorded in the Field Data Sheet. A targeted effort to sample and collect fish within St. Lawrence River, Grasse River, Raquette River, and St. Regis River will occur during the spring, early summer survey period (April – June) for two years (2012 and 2013).

Table 2. Fish Sampling Schematic

Sampling Locations	Year 1 (2012)	Year 2 (2013)	Total
St. Lawrence River	70 Fish (AOC & Outside AOC)	70 Fish (AOC & Outside AOC)	140 Fish
Grasse River	70 Fish (AOC & Outside AOC)	70 Fish (AOC & Outside AOC)	140 Fish
Raquette River*	70 Fish (AOC & Outside AOC)	{70 Fish (AOC & Outside AOC)}	70 Fish
St. Regis River*		{70 Fish (AOC & Outside AOC)}	70 Fish
TOTAL			420 Fish

Note (*) – Adaptive Management via consultation with Region 2 EPA Project Officer (PO) will be used to determine if Year 2 sampling will include the Raquette River or St. Regis River, or both depending on funding availability for lab analysis and data results from Year 1. The USEPA PO requested the inclusion of the St. Regis River since it is one of the four tributaries that make up the St. Lawrence River Massena AOC. Although there is no direct contaminant source in the St. Regis River, there are data gaps. The sampling in Year 2 needs to include St. Lawrence River and the Grasse River because of the proximity to Superfund Sites, and replicable data sets are preferred. In order to delist the AOC, it is important to investigate all 4 waterways which are part of the AOC, and include the St. Regis River. The condition of one waterway is not representative of the whole AOC.

Figure 2. Massena Area of Concern Study Area for Fish Tumors



3. 2 Work Schedule:
April-August 2011 –
Draft QAPP compilation, review, comment period, revisions and Final QAPP completion

April-June 2012
Sample collection of 210 brown bullhead (or yellow bullhead and/or white suckers) will be conducted using netting and electro-fishing. Liver samples (210) will be submitted to qualified fish histopathologist laboratory, and subsample (~40) of fish fillet tissue sent to an approved laboratory for chemical analysis (% lipids, PCBs, and PAHs).

April-October 2012

Liver Histopathology analysis (targeting 210 livers)
Tissue Analysis (targeting 40 fillets)
Summary of External Anomaly Analysis (targeting 210 fish)
Coordinate with Alcoa's Resident Trend Monitoring Fish Collection efforts (September)
Summary of 2012 Fish Tumors or other Deformities Preliminary Results
Submitted to EPA Project Officer in coordination with Semi-Annual Report October2011

April – June 2013
Sample collection of 210 brown bullhead (or yellow bullhead and/or white suckers) will be conducted using netting and electro-fishing. Liver samples (210) submitted to qualified fish histopathologist laboratory, and subsample (~40) of fish fillet tissue sent to an approved laboratory for chemical analysis (PCBs and PAHs).

April-September 2013
Liver Histopathology analysis (targeting 210 livers)
Tissue Analysis (targeting 40 fillets)
Summary of External Anomaly Analysis (targeting 210 fish)
Coordinate with Alcoa's Resident Trend Monitoring Fish Collection efforts (September)
Summary of 2013 Fish Tumors or other Deformities Preliminary Results
Submitted to EPA Project Officer in coordination with Semi-Annual Report October2013

July-September 2013
Comprehensive Fish tumor and tissue analysis Data (for both years of study) included in Fish Status Report Compilation Efforts.

Section 4.0 DATA QUALITY OBJECTIVES FOR MEASUREMENT DATA

4.1 Project Quality Objectives

The specific objective of this study is to assess the prevalence of tumors or other deformities in Massena/Akwesasne Area of Concern fish. The objectives are outlined in Section 2.1. General quality objectives, acceptance and performance criteria for the Massena/Akwesasne Fish Tumor BUI Assessment are summarized below. Appendix C-G provides detailed acceptance and performance criteria for analytical methods.

- Secondary Data or Historical Data Collection:
  - Quality Objective:
    - To include existing data that meets quality objectives for the RAP as per NYSDEC or USEPA standards/procedures, or peer review (i.e. publication).
  - Acceptability/Performance Criteria:
    - Data must be from original source
    - Data generated utilized approved methodologies (See Section 15.0)
- Sampling and Analysis
  - Quality Objective:
    - To have samples and analytical results that accurately represent conditions in the fish from the site.
    - Data must be sufficient quality to meet regulatory requirements and assess comparison to background references.
    - Data must allow for comparison of chemical analyses of fish tissue to reference sites.
    - Field sampling efforts must document activities and comply with this QAPP.
  - Acceptability/Performance Criteria:
    - Data to be collected under an approved QAPP

        - Reporting limits should be comparable literature values from related studies
        - Data to be compared to data collected in reference sites.
- Field Records
    - Quality Objectives:
        - To document all field activities and be representative of accurate field events
        - Reports must be capable of withstanding legal scrutiny
    - Acceptability/Performance Criteria:
        - Clear and legible documentation for sample collection and equipment decontamination for final report
        - Clear and legible documentation for field observation as documented in attached field data sheets.

4.2 Sampling Quality Objectives

Acceptance and performance criteria are often specified in terms of precision, accuracy, representativeness, completeness, and comparability parameters. Numerical acceptance criteria cannot be assigned to all parameters, but general performance goals are established for data collection activities.

Representativeness:

Representativeness expresses the degree to which data represent a characteristic of a population, a parameter variation at a sampling point, or an environmental condition. Representativeness is a qualitative parameter, which is most concerned with proper design of the measurement program. Brown bullhead is used as the indicator species to represent resident fish exposed to contaminated sediment in localized areas throughout the Great Lakes. Although limited data exists on home ranges of bullhead, it is assumed they are localized except in periods of migration, to select representative sites for sampling. Site selection in the Massena AOC utilizes both judgmental selection based on previous exposure sources in the AOC, and suitable habitat for target species (i.e. nearshore areas). Fish selected for sampling will be systematic and random within the site. Every 10^{th} fish from the fish targeted meeting the minimum length requirement (minimum of 250mm) in the study will be submitted for chemical analysis to ensure unbiased representation. The sampling scheme will be conducted similar at both the Inside AOC sampling sites and the Outside AOC sampling sites.

Spatial:

To appropriately delineate St. Lawrence River Massena/Akwesasne Area of Concern (AOC), lines were drawn on a map for representative river stretches in the Grasse River, Raquette River, St.Regis River, and St. Lawrence River according to pre-determined AOC Boundaries (NYSDEC, 1990 Stage I). This area is considered "Inside AOC", while comparable river miles upstream of those stretches is considered "Outside AOC" for Site Selection. In addition to river miles for spatial representation, barriers such as the Madrid Dam on the Grasse River and Hogansburg Dam on the St. Regis River was used to delineate and Outside AOC Sampling Site. Given the uncertainty of the migratory distance of the bullhead, the Outside AOC sampling site selected for the Grasse River and St. Regis River utilizes the first impassable dam on the system to verify non-exposure to the Outside AOC site. The Outside AOC site on the St. Lawrence River utilizes comparable river miles upstream of the AOC, and a location verified in previous sampling efforts (SRMT 2005) to identify an appropriate Massena AOC Reference Location on the St. Lawrence River.

Temporal:

Surveys will be conducted during spring-early summer (April-June), to target collection of fish pre-spawning for reproduction health assessment. The reproductive assessment will follow procedures outlined in Appendix D, Section 6.3, if sufficient time in the field during processing. Observations and

weights of the gonads will be recorded in Field Data Sheet. Gonadosomatic index (GSI) is a ratio of the weight of the gonads to the weight of the fish. This index will be used to compare fish reproduction health collected inside the AOC vs. Outside the AOC each year. This sampling period is also consistent with other Great Lakes AOC Fish Tumor published literature. Two surveys will be conducted to evaluate annual variability.

Fish tissue concentrations or liver histopathology studies will not be compared to toxicity reference values (TRVs) to determine the likelihood of reproductive effects. That was not the objective of the study, but such literature may be reviewed as outlined in Section 15.0. The assessment of the gonads and GSI will be to strictly compare Inside AOC to Outside AOC to demonstrate whether or not our BUI is Impaired according to the delisting criteria outlined in Table 1. "No reproductive deformities in observed resident species as compared to control sites". Observations of the liver and spleen will also be made as supporting lines of evidence of fish health according to Appendix D.

Completeness:
Every reasonable effort will be made to collect the numbers and sizes of fish described in Section 3.0. Based on guidance provided in Appendix C, Rafferty and Grazio 2006, Section 5.4 i.e., "30-50 brown bullhead should be randomly selected for histopathology from each sampling location, if possible". Following this guidance, 35 fish are considered acceptable for a complete sampling. As few as 30 fish per sampling site would be considered acceptable, with more preferred for statistical significance. If after extensive collection efforts via netting and/or electro fishing numbers and sizes of fish cannot be obtained, SRMT BUI Coordinator will consult with USEPA Project Officer, Barbara Belasco for mutual agreement on course of action. Possible courses of action might include collection of fish in adjacent areas, collection of smaller fish, or collection of less fish.

Comparability:
Comparability is a qualitative parameter expressing the confidence with which one data set may be compared to another. Sample data should be comparable with other measurement data for similar samples and sample conditions. This goal is achieved through the use of standard techniques to collect and analyze samples. Historical data will be evaluated to ensure the methods and reporting limits are comparable to the proposed sampling. Secondary Data will only be evaluated if it is determined to be comparable and appropriate according to Section 15.0.

Samples collected Inside the AOC will be compared to samples collected Outside the AOC for measurement quality objectives consistent with the Delisting Criteria, and USEPA procedures.

Precision
Precision measures the reproducibility of measurements under a given set of conditions. Specifically, it is a quantitative measure of the variability of a group of measurements compared to their average value, usually stated in terms of standard deviation or coefficient of variation. It also may be measured as the relative percent difference (RPD) between two values.

The most commonly used estimates of precision are the relative standard deviation or coefficient of variation (CV) for multiple samples, and the relative percent difference (RPD) when only two samples are available. These are defined as follows:

$$RSD = CV = 100\frac{S}{\overline{X}_i}$$

Where

S = Standard deviation of the X_i measurements

X_i = Arithmetic mean of the X_i measurements

And

$$RPD = 100\left\{\frac{(X_1 - X_2)}{(X_1 + X_2)/2}\right\}$$

For this project the target RPD/RSD is 15% for acceptance of data. The Project Manager will select the sample(s) for additional duplicate analysis. The original sample with be analyzed and additional sub-sample will be taken from the homogenate and analysis separately.

Accuracy
Accuracy measures the bias of the measurement system. Sources of this error are the sampling process, field contamination, preservation, handling, sample matrix, sample preparation, and analysis. Data interpretation and reporting may also be significant sources of error. Typically, analytical accuracy is assessed through the analysis of spiked samples and may be stated in terms of percent recovery or the average (arithmetic mean) of the percent recovery. Blank samples are also analyzed to assess sampling and analytical bias (i.e., sample contamination). Background measurements similarly assess measurement bias. The number of samples collected will impact the confidence of the statistical data evaluation. Archive samples will be collected for analysis if additional samples are required.

Section 5.0 SPECIAL TRAINING REQUIREMENTS

All project personnel will be provided the QAPP for review prior to project start-up. The field team will hold a project kick-off meeting prior to start of sampling to review procedures. The Laboratory Project Managers (both chemical and histopathological) and SRMT Project Manager will oversee the implementation of the QAPP in the laboratories.

The field sampling crew has been selected based on experience and already having the necessary knowledge to perform field activities. This includes the experience and ability to identify habitat and locations to collect sample fish from, work with hoop nets, perform visual observations, and record external abnormalities with brown bullhead, and to prepare samples for laboratory analysis. Field crew will use the proper techniques with the sampling gear, sample collection procedures and documentation for QA/QC in the field. The field crew leader will be Jay Wilkins who will make field decisions based on his experience with the St. Lawrence River AOC. Jay will work in consultation with NYSDEC Fisheries

Biologist Rodger Klindt and SRMT Fisheries Technician Jim Snyder. Rodger Klindt will be operator of the electro fishing boat and lead all tasks related to electro fishing operations. Prior to electro fishing sampling, SRMT personnel would be given a safety briefing to include; general operation of the boat, systems (outboard, generator, electrical), electro fishing theory (brief), safety measures, emergency shutdown, and location and use of the AED on board. SRMT will not be operating the electro fishing boat; the representative will assist in collection of fish and processing of the samples only. NYSDEC will follow their Standard Operating Procedures for Electro fishing Safety.

Laboratory project managers will be qualified personnel, as demonstrated in ELAP certification for chemical analysis and professional certification evidence and experience for fish histopathology work (Appendix F).

Section 6.0 DOCUMENTATION AND RECORDS

6.1 Field Record Keeping

Field data entry will be conducted using data sheets (see Appendix C & D). Data will be subsequently entered into a project database using Access. Supplemental field notes will be made in a field journal. All entries will be made in ink, signed or initialed, and dated. No erasures will be made. If an incorrect entry is made, the information will be crossed out with a single strike mark that is signed or initialed and dated by the sampler. Photographs will be numbered sequentially and label ID visible in photograph. A brief description regarding the photograph will be noted. The use of photography will be employed to record field sampling activities and to support documentation of gross visual fish observations. Rafferty and Grazio 2006 provide details on photographic procedures for gross fish observations. The sequential number of the digital photo information will be noted on the pertinent datasheets concerning photographs. Each photograph will be taken with the Sample ID located in the photo frame. All Field data sheets, analytical reports, field journals will be maintained by the Wildlife Technician, and retained in a secure area within SRMT Environment Division for up to five years after the final report is issued.

6.2 Location ID and Sample ID

Samples will be identified using the format described below. There will be two types of fish tissue samples collected for laboratory analyses: fish tissue chemistry (fillet) and liver histopathology. Each sample will be labeled, chemically preserved (as required), and sealed immediately after collection. To minimize handling of sample containers, labels will be completed prior to sample collection as practicable. Adhesive sample labels will be completed using waterproof ink and will be firmly affixed to sample containers and protected with additional clear tape. The sample label will give the following information:

- Date of collection;
- Location of Collection
- Unique Sample ID;
- Analyses requested; and
- Preservation

Each sample will be referenced by sample number on respective data sheets, field logbooks, and on the chain-of-custody (COC) record.

Individual samples will be identified by a unique alphanumeric code. Field Sample ID will be numbered according to the following convention:

SLR2011##-BB

SLR - Three letter code for site name (see below)
(R) - to indicate upstream reference locations
2011 or 2012- Year of Survey
- Sequential sample number (01, 02, 03, etc. up to 35)
BB - Species collected (BB – brown bullhead, YB – yellow bullhead, OSP – other species (to be cross-referenced to survey datasheet)

Site code will represent river abbreviation sample collection was from, and inclusion of (R) if from an upstream outside AOC reference location.

SLR – St. Lawrence River (i.e. SLR-(R) for outside AOC)
GRA – Grasse River (i.e GRA-(R) for outside AOC).
RAQ – Raquette River (i.e. RAQ-(R) for outside AOC)
STR - St. Regis River (i.e. STR – (R) for outside AOC)

6.3 Chain of Custody

A Sample Identification label will be attached to all sample vials and bags using a clear tape sample label that includes sampling date/time, Location ID, Sample ID, fish species collected, sample type, and chemical to be analyzed. Bullhead samples will be sent to the laboratory for chemical analysis as untrimmed fillets, and sent to the histopathology laboratory as liver sections preserved in 10% Formalin, secured with tamper proof seals. A Chain-of-Custody (COC) Form provided by the Laboratory will be filled out and included in a sealed plastic bag taped to the top of the cooler (Appendix H). Fish fillet samples will be double wrapped in hexane rinsed aluminum foil, secured in a ziploc bag and packed on 12-hour ice packs to be sent via Fed-ex to the lab. Taping the COC to the top of the cooler is a preventative step to ensure the form does not get wet and compromise the written documentation. Fish sample retention time if frozen is 1 year, but a 10 day turnaround time is requested.

6.4 Laboratory Data Reporting

The laboratory will provide an electronic data deliverable that matches all data reported on the hard copy analytical report. The analytical summary report will include the sample aliquot analyzed, final extract volume, and dilution factor. The analytical summary data report also will include the laboratory reporting limit and method detection limit (MDL) for all target compounds. These limits will be corrected for percent moisture and all dilution factors. Any compounds found less than the reporting limit, but greater than the MDL will be reported and qualified with a “J” flag as estimated.

QC reports will provide a summary report or batch identifier clearly linking all QC results to actual field sample results. QC summary reports will include the laboratory control limits and flag any result reported outside control limits. The case narrative must include an explanation of all QC results reported outside control limits. The laboratory must provide copies of any nonconformance or corrective action forms associated with data in the laboratory report. Standard laboratory turn-around time for chemical reporting is 10 days.

Histopathology reporting will be conducted according to Appendix E, and delivered to SRMT Project Manager as an Interim Pathology Report after Year 1, and comprehensive Pathology Report synthesizing results from Year 1 and 2 sampling efforts.

Section 7.0 SAMPLING PROCESS DESIGNS

7.1 Sampling Design

The purpose of the sampling described in this section is to collect data necessary to evaluate the status of fish tumors and other deformities BUI in the Massena AOC (status unknown).

In order to assess the status of Fish Tumors BUI, sampling and analytical processes have been developed to collect brown bullheads (or other bottom-dwelling fish if the bullhead is not available) for gross external and internal visual observations for lesions, tumors, ulcers, etc, ; liver pathology; and tissue chemistry (PCBs and PAHs) to determine the presence of chemical exposure within the study area and quantify and report differences between Inside and Outside AOC.

The sampling design was set up to spatially represent comparable areas of the Massena/Akwesasne Area of Concern to make a definitive assessment of comparable incidence rates of fish tumors "Inside" and "Outside" the AOC, to be site specific within the St. Lawrence River Watershed.

Using the home range and preferred habitat of the target species, six (6) sites are targeted for collection of 105 fish inside AOC and targeted for collection of 105 fish Outside AOC (Figure 2) Based on the home range of the target species and suitable habitat availability extending a collection effort will target the shoreline of up to 4 km in length to collect 210 fish for gross external and internal histopathology assessment. 20% of these fish will be sent to the laboratory for homogenization, % lipids, and PCB Aroclor and PAH analysis. Additional weight measurements will be made of the liver and gonads (time permitting) to provide an index of fish health and reproduction health to be used as additional lines of evidence for delisting criteria objectives. Hepatosomatic Index (HSI) and Gonadosomatic index (GSI) is the proportion of the organ weight to the total body weight, expressed as a percent. Enlarged liver organs can occur in fish exposed to trace metals and organic pollutants. HSI has also been related to the presence of liver lesions because the density or mass of affected livers is often increased in response to preneoplastic or neoplastic conditions (http://www.epa.gov/glnpo/arcs/EPA-905-B94-002/B94002-ch8.html#RTFToC46). GSI is a commonly used index to measure the sexual maturity of a fish. Evidence has shown that GSI and testis weight in fish from contaminated systems have been significantly lower during both pre-breeding and post-breeding systems. Reduced gonads can occur in fish exposed to pollutants. (http://snhsplin.barry.edu/Research/Hassanin_estrogen_gonadosomatic_index_carp_2002.pdf). The HSI and GSI will be additional tools used to assess the health and reproductive health of the fish targeted for collection and dissection.

The primary purpose of this sampling event is not to determine the presence of chemical exposure, but rather the presence/absence of neoplastic or preneoplastic liver tumors and external anomalies. It is assumed that "inside the AOC" fish are exposed, and not exposed "outside the AOC". During Remedial Investigation and Feasibility Studies (RI/FS) for the CERCLA sites that make up the AOC, sediment investigation has occurred to delineate area of impact. To adequately answer the delisting criteria targets, sediment samples are not needed.

7.2 Species Sampled

The ability to accurately and consistently identify tumors or other deformities in brown bullhead is critical for proper assessment and monitoring of the status of this BUI.

To determine the prevalence of tumors gross visual external and internal observations and histo-pathological examinations will be conducted to identify potential lesions and neoplasms. The histo-pathological work will involve the examination of the fish livers. This type of examination is a reliable tool for evaluating tissue damage resulting from contaminated sediments and environmental pollution. The target fish species, brown bullhead, will be collected within the Massena Area of Concern at six sites for external gross observations, and liver histopathology analysis and chemical analysis.

Approximately thirty-five (35)adult brown bullheads will be collected within identified sampling sites from the St. Lawrence River, Grasse River, and Raquette River (total of 210 fish) to evaluate the presence of liver neoplasm, preneoplasms, and external lesions and deformities. The sample size was determined based on recommendations of a minimum of 30-50 brown bullhead randomly selected from each sampling location. The recommendation is from Section 5.4 of the Field Manual for Assessing Internal and External Anomalies in Brown Bullhead (*Ameiurus nebulosus*) (Rafferty and Grazio, 2006) ; see Appendix C. The recommendations were developed as part of a series of workshops on the "Development of Standardized Criteria for the Assessment of Brown Bullhead Lesions and Deformities in Areas of Concern". The workshops are cosponsored by: Pennsylvania Sea Grant, United States Environmental Protection Agency Great Lakes National Program Office, and Pennsylvania Department of Environmental Protection (see http://seagrant.psu.edu/publications).

At the end of each electro fishing run (or subsequent to retrieval of nets), fish will be processed by identifying each specimen, recording length (total length—TL to nearest mm) and weight (grams) using a digital top-loading scale. Identification of specimens contained in the samples will be to the lowest practicable taxonomic level using one or more of the following taxonomic keys:

Smith, L. 1985. *The Inland Fishes of New York State*. The New York State Department of Environmental Conservation. Albany, NY.

Kraft, C. E., D. M. Carlson, and M. Carlson. 2006. *Inland Fishes of New York (Online)*, Version 4.0. Department of Natural Resources, Cornell University, and the New York State Department of Environmental Conservation.

Field Data Collection
Field data collection activities will be recorded using project specific field data sheets; similar information will be collected in the field during the targeted fish collection activities. For each sampling event, the following field data will be recorded:

- General Sample Identification
  - Date
  - Start/Stop time
  - Weather
  - Field Crew
  - Sample Collection Method
  - Sample Location
  - Sample Identification

- Biological Data
  - Species common name
  - Length (nearest mm)
  - Weight (nearest g)
  - Status/condition (live, dead, moribund)

- External Disease, Erosion, Lesions, Tumors (see Section 5.3 Appendix C and Data Sheets)
- Heptosomatic Index (HIS): calculation of percent ratio of liver wet mass to total body wet mass: [(liver wt/body wt)*100] as per USGS 2002.
- Gonadosomatic index (GSI): calculation of percent ratio of gonad wet mass to total body wet mass: [(gonad wt/body wt)*100] as per USGS 2002.
- Sex (see Appendix C)
- Age (Otolith and Spine; see Section 5.5 Appendix C)

Section 8.0 SAMPLING METHODS REQUIREMENTS

8.1 Field Sampling

The proposed gear types will include electro fishing, and hoop netting, and potentially angling. Hoop netting will be the primary gear type and will be used at selected locations along the shoreline within each study reach. Electro fishing shall be performed using a boat-mounted Smith Root pulsed DC electro fishing for 15 minute durations at each location. Fish immobilized during each electro fishing run will be dip-netted and put into aerated live wells for processing.

All sampling areas will be mapped using handheld GPS. Hoop nets (one-meter diameter) will be used opportunistically within each sampling reach to collect additional fish specimens, or when electro fishing equipment support by NYSDEC is not available. Fish collected by netting shall be removed from net cod ends, and placed in an aerated live well for processing. Net set time and run times shall be recorded for each net location, in order to calculate catch per unit effort. Additionally ancillary information that shall be recorded for net samples shall include water depth, in-stream habitat characteristics, and relative water flow characteristics.

8.2 Targeted Fish Collection for Histopathology

The primary collection method will be hoop netting. The prevalence of deeper water habitats and lower-than-anticipated numbers of fish collected via electro-shocking will require the use of hoop nets. If an insufficient number of fish are collected, sampling may include: bag seines, and/or angling. If an insufficient number of brown bullhead is collected, other Ictalurids (yellow bullhead) and possibly Catastomids (white sucker) will be collected for analysis.

Subject fish will be kept alive during hoop net collections and electro fishing runs, up to the point of processing individual fish. The literature indicates that fish that die prior to being assessed should neither be grossly assessed or necropsies due to the potential for the development of post-mortem lesions (Section 4.4, pg. 5 of Rafferty and Grazio, 2006). Gross internal, external, and liver and fish tissue sample processing will occur streamside, or at SRMT laboratory space (weather dependent) after each electro fishing/sampling run in order to minimize the possibility of fish mortality.

Gross internal and external visual observations will follow the procedures outlined in Section 5.3 of the Field Manual for Assessing Internal and External Anomalies in Brown Bullhead (*Ameiurus nebulosus*) (Rafferty and Grazio, 2006, pg. 7-13); see Appendix C. Fish Health Data will be reported in the Field Data Sheet in Appendix C below. Photographs will be taken of all fish exhibiting tumors, lesions, or other deformities, with the appropriate labeling (sample collection date, location, species, etc.) shown next to the fish for photo-documentation. For preparing the livers for histo-pathological examination, livers will be excised from each fish and laid flat on a cutting surface. Five transverse slabs, each less than 1 cm thick (5-7mm), will be trimmed from each liver. The slabs will be located approximately equidistant from

one another, except that one or more of the slabs may be oriented to include any macroscopic liver lesion(s) that might be present. The slabs will be placed immediately into the fish's labeled individual container of 10% neutral buffered formalin. There will be sufficient formalin in each container so that the volume of fixative is at least 10 times the volume of the tissues. Portions of any non-hepatic tissues that have macroscopic abnormalities (those that are amenable to sampling) may also be placed in the same formalin container; these specimens will be trimmed so that they are no wider than 1 cm in at least one dimension, and they should contain some of the adjacent normal tissue if possible. Such specimens may be placed into labeled tissue cassettes to facilitate subsequent identification. No other tissues will be collected routinely unless specified by protocol amendment. The additional samples could be used for analysis under a future project but is not considered within the scope of the existing project.

Unless otherwise specified in Appendix D & E, all histo-pathological procedures will be performed according to Cornell University Laboratory's Standard Operating Procedures. Cornell University, College of Veterinary Medicine was chosen based on performance, experience, qualification certifications, and cost reasonability. Dr. Paul R. Bowser, Professor of Aquatic Animal Medicine has over 25 years as Associate Director of AQUAVET Program, instruction, and fish pathology work. Dr. Paul R. Bowser has ready access to consultation with the Section of Anatomic Pathology of the College of Veterinary Medicine at Cornell University (Appendix F).

Each of the five liver slabs will be trimmed transversely (i.e., perpendicular to the long axis of the tissue as submitted) to provide at least one flat surface for microtoming, and so that the trimmed specimen can be placed into a standard tissue cassette. The remaining liver tissues will be retained in the animal's individual formalin container. Liver slabs with obvious lesions will be trimmed so that a portion of the lesion(s), and, if possible, a portion of adjacent unaffected tissue, will be evident in the single microtomed section to be produced from each slab. Excluded from this requirement are lesions that are clearly parasitic in origin based on macroscopic observation. Specimens in cassettes will be processed to paraffin-embedded sections on glass slides according to routine methods, and the slides will be stained with hematoxylin and eosin prior to coverslipping. Non-hepatic tissues may be retained in the animal's individual formalin container and not processed to slides unless specified by protocol amendment or other written directive from the SRMT Project Manager. Similarly, as above, these tissues may be collected for future analysis under a different project.

Each of the sections on glass slides will be examined via light microscopy by a fish pathologist who has experience in the evaluation of neoplastic fish diseases. During the initial histo-pathologic evaluation, the pathologist will be aware of the collection site status of each animal, as advocated in Crissman et al., 2004. Unless otherwise specified, proliferative liver lesions (foci of cellular alteration and primary liver neoplasms) will be assessed according to criteria and terminology described in Blazer et al., 2006. If multiple proliferative lesions of a single type are present in one section, these will not be quantified; however, in such instances the term "multiple" will be a component of the diagnosis. In general, non-proliferative lesions will be reported and scored for severity according to the following grading scheme: 1 = minimal, 2 = slight/mild, 3 = moderate, 4 = severe. Altered foci and neoplasms will not be scored for severity, and instead will be reported as "Present". Certain types of non-proliferative lesions that are not amenable to severity scoring may also be reported as "Present". The pathologist will attempt to correlate macroscopic observations made at necropsy or gross trimming with histopathologic diagnoses. Diagnoses will be recorded into an electronic data system for tabulation and reporting. The pathology report will include, but not limited to: a narrative pathology summary (Introduction, Methods, Results, Discussion, Summary and Conclusion sections); Histopathology
Incidence Tables (HIT) and Summary Incidence Tables (SIT); and Correlation of Gross and Histopathologic Findings tables.

[See Blazer, Fournie, Wolf, and Wolfe (2006) and Crissman, Goodman, Hildebrandt, Maronpot, Prater, Riley, Seaman, and Thake (2004) for supporting material regarding histo-pathological procedures.]

Field Technician Steps (Following Appendix C-E):

1. Fish will be sampled in the spring because that is when the bullheads are most abundant and easiest to capture, to eliminate unnecessary man-hours in the field for large number of sample size
2. Due to a latent period between exposure to contaminants and tumor incidence, fish < 3 years of age rarely display tumors. Therefore, field sampling efforts will target fish at least 3 years of age and older. For bullhead, it is widely accepted that fish 250 mm in length are at least 3 years old. A population of sufficiently older fish is needed for comparison purposes. Length will be used only as a field tool for targeting number of fish. Ages will be confirmed for data analysis utilizing otolith and/or spine aging techniques.
3. Fish will be collected systematically from field sites to eliminate sampling bias, and maintained in a live well until processing.
4. Sample IDs, sample labels, photo-labels, and Field Data Sheets will be filled out as complete as possible prior to fish processing, or completed by one technician while the other technician is performing processing procedures. The second field technician will verify all observations.
5. External Observations and Gross Anomalies to be conducted based on Appendix C.
6. Field Technicians to document location and severity of lesion(s) and anomalies as per Field Data Sheet (Appendix C).
7. When noting external anomalies, include ulcerated lesions and melanosis in gross observations, but do not take a sample of these lesions. Ulcerated lesions and melanomas are not suspected to be a result of contamination; however, they could be a result of an immune deficiency problem that is caused by exposure to contamination.
8. When external observation is complete proceed to Euthanizing procedures (See Section 5.4.1 in Rafferty and Grazio, 2006, Appendix C).
9. Proceed to follow Sections 5.4.2-5.4.4 in Rafferty and Grazio 2006, Appendix C for Lesion and Liver Biopsy.
10. If the external lesions are raised (including ulcerations and melanistic areas), take a single section approximately 5-7mm in width through the lesion from the largest lesion present. Be sure to include adjacent and underlying tissue in the sample. If the lesion is small (less than 5 mm) take the entire lesion. If it is extremely large, take a section from one edge of the lesion.
11. After exposing the liver by opening the abdominal cavity, follow procedures to remove the entire liver and gallbladder as per Section 5.4.4 in Appendix C.
12. Then remove the gallbladder where it attaches to the liver to prevent puncturing the gallbladder; bile from the gallbladder, can contaminate the liver tissue, causing it to be unusable. If the gallbladder is punctured, run the liver under water before putting it into fixative.
13. The gallbladder is not needed in samples for histopathology, and can be disposed of. Prior to disposal, record notes of observations of bile in Field Data Sheet.
14. Weight the entire liver to the nearest 0.1 gram
15. Do not put the whole liver into fixative. Using a sharp blade (disposable microtone blade or #22 blade) on a flat board, make five cross sectional cuts 5-7mm in thickness working from anterior to posterior. Take care not to mash the liver down. If a grossly observable lesion is present on the liver be sure to include it in one of the cross sections.
16. If time permits, make observation notes of the gonads and spleen and record in Field Data Sheet. Weigh gonads to 0.1 gram for GSI index.
17. Use 10% formalin as fixative (approximately 10 to 1 fixative to tissue sample ratio). Fixative will be changed every 12 hours. Package/shipping to lab for histopathological analysis using 4-6um thick slices.

18. Extract both a spine and otolith for aging and comparison following Section 5.5 in Appendix C. Archive spine and otolith for aging analysis by NYSDEC and SRMT Technicians at a later date (prior to data analysis).
19. Remove fillet samples for chemical analysis on every 10th fish processed for histopathology. Sample will be filleted, creating split samples (A) and (B). (A) will be analyzed immediately and (B) will be saved as backup, stored at the SRMT Environment Division.
20. Fillet will include the skin, belly and dorsal fat. Both fillets are to be wrapped in separate hexane rinsed aluminum foil.
21. The fillet will be placed in a labeled plastic bag; the fillets will be sealed in closable plastic bags and stored at -20˚C for shipping and Chain of Custody procedures.
22. Field Technician to complete field data sheets, COC, and clean-up and decontamination steps.

8.3 Fish Tissue Chemistry

Twenty bullheads (9 to 12 inch length) from both the AOC and background area will be collected for PAH and PCB analysis (see Table 1-3). This sample size will be adequate to detect a 50% increase over background with a statistical power and confidence of 90%, assuming a coefficient of variation (CV) of 80% for the fish contaminant data (see Table3). As a contingency, SRMT will process both fillets of the fish from both the AOC and background area, complete with sampling labels. One fillet will be sent to the laboratory for chemical analysis, and the second one will be archived in a freezer at SRMT. After the data has been received and evaluated, the fate of the archived fish tissue will be determined.

Table 3. Relationship Between Measures of Statistical Performance and Number Of Samples Required

Coefficient of Variation (%)	Power (%)	Confidence Level (%)	30%	50%	100%
10	90	90	2	1	0
20	90	90	4	2	1
30	90	90	8	4	1
40	90	90	14	6	2
50	90	90	20	8	3
60	90	90	28	11	4
70	90	90	38	15	5
80	90	90	49	19	6
90	90	90	61	23	7

Notes:
1. Based on EPA (1989, 1992).
2. Number of samples required to identify differences of 30%, 50%, and 100% over background

Section 9.0 SAMPLE HANDLING AND CUSTODY REQUIREMENTS

Sample Handling and Shipping

All fish samples will be preserved on ice immediately after collection. The samples for fish livers will be prepared as noted in Appendix C and preserved with 10% buffered formalin. The remaining fish tissue will packaged on ice and shipped to the laboratory.

The laboratory will either freeze the fish or process immediately. There are no specific holding times for fish tissue. As general guideline tissue can be analyzed within one year of freezing.

The transportation and handling of samples must be accomplished in a manner that not only protects the integrity of samples but also prevents any detrimental effects due to the possible hazardous nature of the samples. Regulations for packaging, marking, labeling, and shipping of hazardous materials are promulgated by the DOT in 49 CFR 171, 172, 173, and 177. To facilitate shipping of Formalin samples, Cornell University will utilize its Aquatic Animal Program to send a Fleet Vehicle to the St. Regis Mohawk Tribe to collect and transport samples preserved in 10% neutral buffered formalin at least 2x's/sampling year.

Sample Packaging

Samples must be packaged carefully to avoid breakage or contamination and must be shipped to the laboratory at proper temperatures. Samples for chemical analysis will be packed according to NEA-PACE instruction, and samples for liver microscopy work will be packaged in accordance to Appendix D & E.

Sample Custody

Formal sample custody procedures begin when the samples are collected. The laboratory must follow written and approved SOPs for shipping, receiving, logging, and internally transferring samples. Sample identification documents must be carefully prepared so that sample identification and Chain of Custody (COC) can be maintained and sample disposition controlled. Sample identification documents include:

- Field data sheets;
- Sample labels;
- Custody seals; and
- COC records.

The primary objective of COC procedures is to provide an accurate written or computerized record that can be used to trace the possession and handling of a sample from sampling through completion of all required analyses. A sample is in custody if it is:

- In a team member's physical possession;
- In a team member's view;
- Locked up; or
- Kept in a secured area that is restricted to authorized personnel.

Field Custody Procedures

The following field custody procedure will be used for collection of samples:

- As few persons as possible should handle samples;

- ❖ Coolers or boxes containing cleaned bottles should be sealed with a custody tape seal during transport to the field or while in storage prior to use;
- ❖ The sample collector is personally responsible for the care and custody of samples collected until they are transferred to another person or dispatched properly under COC rules;
- ❖ The sample collector will record sample data in the field data sheet; and
- ❖ The Field Team Leader will determine whether proper custody procedures were followed during the fieldwork and decide if additional samples are required.

Chain-of-Custody Record
The COC form must be fully completed in duplicate by the field technician designated by the Project Manager as responsible for sample shipment to the appropriate laboratory for analysis. In addition, if samples are known to require rapid turnaround in the laboratory because of project time constraints or analytical concerns (e.g., extraction time or sample retention period limitations), the person completing the COC record should note these constraints. The custody record also should indicate any special preservation techniques necessary or whether samples need to be filtered. Copies of COC records are maintained with the project file by the Field Crew Leader.

Custody Seals
Custody seals are preprinted, adhesive-backed seals with security slots designed to break if the seals are disturbed. Custody seals are placed over the cap of individual sample bottles by the sampling technician. DOT-approved sample shipping containers are sealed in as many places as necessary to ensure security. Seals must be signed and dated before use. Upon receipt at the laboratory, the custodian must check and document on a cooler receipt form that seals on boxes and bottles are intact.

Sample Acceptance and Logging
Coolers shipped to NEA-PACE will include an internal bar coded label, fixed to the inside of the cooler that can be traced by the laboratory and imported into their Laboratory Information Management System (LIMS) to track sample delivery groups (SDG) and analytical results with an associated Laboratory Record File (LRF). The LIMS system generates an automated email notification of SDG, and reports on the condition of the received sample group. The SRMT Project Manager will review this sample receipt report within 48 hours for completeness and accuracy, and work with the Project Chemist if change orders and or corrections are needed.

Section 10.0 ANALYTICAL METHODS REQUIREMENTS

The selected laboratory to perform the analyses is Northeast Analytical, A Division of Pace Analytical Services (NEA-PACE). It is the responsibility of the laboratory to certify the validity and accuracy of the data they submit, within the parameters of the methodology they utilize or may have been specified below. The Project Chemist will take appropriate corrective actions as needed, and coordinate with the SRMT Project Manager. Analyses and reports shall be provided in Summary Reports and Electronic PDF, and EDD. SOPs are provided for PCBs by Method 8082A (NE148) and PAHs by EPA 8270C (NE238). Additional SOPs for percent lipid (NE158) determination and fish sample preparation (NE 132 and NA05) also are provided (Appendix G).

Fish Analysis:

PCB Analysis: EPA 8082
PCB MDL: 0.0104 mg/kg

PCB PQL: 0.050 mg/kg
TAT:` 10-15 working days

PAHs Analysis: EPA 8270C
PAHs MDL: 0.033 mg/kg
PAHs PQL: 0.100 mg/kg
TAT: 10 - 15 working days

The Laboratory will perform tissue prep grinding and homogenization, utilize EPA Method 3540 for fish extraction by Soxhlet, and utilize EPA Method 3640 as needed for lipid preparation for GC/MS analysis of PAHs.

-Technical Holding Time: 1 year (when kept frozen for sample to still be considered valid)
-Extraction Holding Time: 2 weeks (time allowable to extract sample once thawed)
-Contractual Holding Time: 40 days (time allowable between extraction and analysis)

NEA-PACE laboratory was chosen based on past performance history and experience, the precision and accuracy in their performance methods, and repeated years of qualification certifications. NEA-PACEis certified by the New York State Department of Health and participates in the Environmental Laboratory Approval Program (ELAP) and the USEPA WP QC Program. NEA, Inc. adheres to the strict QA/QC program outlined in the ELAP manual. Laboratory selection justification is based on:

- Performance Methods
- Experience and Past Performance History
- Statement of Certification and Audits
- Cost Reasonability

Section 11.0 QUALITY CONTROL REQUIREMENTS

11.1 Field

Data Quality is assured through the use of experienced and knowledgeable field crew, care taken in observation and documentation procedures, and following methodologies and Safety Plan of Action. The SRMT QAMP will be followed as a management tool for planning, implementing, documenting, and assessing the effectiveness of activities supporting environmental data operations (submitted electronically to EPA previously).

Experienced field personnel assure the accuracy of identification of target and non-target fish species. The field crew is responsible for ensuring all sampling gear and equipment is in good working condition and signing off on the Field Supplies Checklist (Appendix A).

Photographs are taken with a 6-inch ruler and photo ID. Fish are measured to the nearest mm, and recorded to the nearest gram (g). When weighing liver and/or gonads, measurements are recorded to the nearest tenth of a gram (0.1 g). External abnormalities are assessed using a scoring index, and all details are recorded in a Field Data Sheet. Field Crew Leader is responsible for all proper documentation in the Field Data Sheets and Field Log Book as described in Section 6.0, and signs off on them indicating their review and approval. Latitude and longitude coordinates are recorded for each sampling site using a handheld Magellen eXplorist 610 Global-Positioning systems (GPS) to confirm sampling location, and distance traveled (if using electro fishing methods). The handheld GPS has an accuracy of +/- 3 meters is only used as a verification tool to estimate home range of targeted fish, and is not critical for Location ID positioning. NYSDEC Fisheries Biologist and SRMT Field staff is familiar with proposed site locations, and river navigability. Pre-determined site sampling distances will be assessed using Google Earth.

The Project Manager will review all field data sheets and documentation for any discrepancies and will be responsible to resolve those discrepancies.

11.2 Laboratory Quality Control Analyses

QC data are necessary to determine precision and accuracy and to demonstrate the absence of interferences and/or contamination of glassware and reagents. No specific field QC samples are required for tissue samples. Laboratory-based QC will consist of standards, replicates, spikes, and blanks. Method QC limits for analyses are provided in Table 4 & Table 5.

Analytical performance is monitored through QC samples and spikes, such as laboratory method blanks, surrogate spikes, QC check samples, matrix spikes, matrix spike duplicates, duplicate samples, and duplicate injections.

- One Method Blank (MB) per matrix per preparation batch for each analysis
- One Matrix Spike Blank (MSB) per preparation batch for each analysis.
- Surrogate Spikes for all samples analyzed for organic methods.
- One Matrix Spike/Matrix Duplicate (MS/MD) for each analysis. The spike solution must contain a broad range of analytes of concern at the site. The overall frequency of MS/MSD on project samples must be at least one per 20 samples.

Two extraction lab technicians will set up the 20 samples plus QC (Lab Blank, Lab Control Spike, and other field required QC samples) into Soxhlet. Sample extraction batches will be 20 samples or less, depending on what is being received from field collection efforts. If more than 20 samples are received on a given day, samples will be batched in extraction batches of 20. When possible, the remaining samples will be held and combined with the next day's shipment. This system will provide the most efficient QC batching.

Instances may arise where high sample concentrations, nonhomogeneity of samples, or matrix interferences preclude achieving detection limits or associated QC target criteria. In such instances, data will not be rejected *a priori* but will be examined on a case-by-case basis. The laboratory will report the reason for deviations from these detection limits or noncompliance with QC criteria in the case narrative.

Table 4. QC Requirements for Method 8082 (PCB Analysis)

Quality Control Item	Frequency	Acceptance Criteria
Method Blank	One per extraction batch of ≤20 samples of the same matrix per day	-Concentration does not exceed the Reporting Limit for any PCB Aroclor -Meets 60-140% recovery
Matrix Spike/Matrix Spike Duplicate (MS/MSD)	One MS/extraction of a batch of ≤20	-% recovery for MS must be within method limits -Must meet Aroclor spike criteria of 70-130% recovery -Must meet surrogate criteria of 60-140%
Surrogates	Surrogates are added to all samples and QC samples. Tetra-chloro-meta-xylene (TCMX) and Decachlorobiphenly (DCBP) solution	-Percent recover for the surrogate should be 60-140%

Table 5. QC Requirements for Method 8270 (PAH Analysis)

Quality Control Item	Frequency	Acceptance Criteria	Corrective Action
Method Blank	One per extraction batch of ≤ 20 samples of the same matrix per day.	The method blank concentration should be less than the Practical Quantitation Limit/Reporting Limit for each analyte of interest.	Re-analyze method blank to determine if instrument contamination was the cause. If method blank re-analysis passes, then report samples. -If method blank is found to contain contamination above the PQL/RL for analyte of interest then re-extract and re-analyze all associated samples. If no sample exists for re-extraction, report data B flagged to indicate method blank contamination.
Surrogate Spike	Each extracted sample, and QA sample including Method Blanks and LCS/LCSD prior to extraction.	The surrogate % recovery for 2 out of 3 of surrogates must be within acceptance limits.	Re-inject sample if indicated. Inspect chromatogram for matrix interferences. Perform instrument maintenance if indicated. Re-extract affected samples. If re-extraction is not possible, report data with sample delivery group case narrative. Repeat failures of surrogate recovery suggest a systematic problem with the extraction/analysis that must be corrected before analysis may continue.
Laboratory Control Spike/Laboratory Control Spike Duplicate (LCS/LCSD)	One set per extraction batch of ≤ 20samples per matrix per day	The LCS/LCSD % recovery should be within lab-established limits for all analytes.	RE-analyze LCS to determine if instrument was the cause. IF LCS passes, then report samples. -If LCS recovery is still out of limits, then re-extract and re-analyze all associated samples. If no additional sample exists for re-extraction, report data flagged with SDG narrative to indicate failed LCS recovery.

Laboratory Method Blank

Laboratory method blanks serve to demonstrate a contamination-free environment in the laboratory. The goal is for method blanks to be free of contamination. Low-level contamination may be present, but must be less than the level in samples as defined by the method SOP. If contamination is greater, samples are

analyzed. If contaminants are present in the method blank but not in project samples, no further action is required. All sources of contamination that are not common laboratory contaminants as defined in the method SOPs must be investigated as part of the corrective action process. Sample results must not be blank subtracted unless specifically required by the analytical method.

Surrogate Standards
Surrogate recoveries must be within QC criteria for method blanks and LCSs to demonstrate acceptable method performance. If surrogate recoveries are outside QC criteria for method blanks or LCSs, corrective action is required and the Project Chemist should be notified. Surrogate recoveries in the samples indicate the method performance on the particular sample matrix. Surrogate recoveries that are outside QC criteria for a sample indicate a potential matrix effect. Matrix effects must be verified based on review of recoveries in the method blank or LCS, sample reanalysis, or evaluation of interfering compounds.

Laboratory Control Sample
LCS recoveries must be monitored on control charts for all non-Contract Laboratory Procedure methods. The LCS recovery must be within the control limits to demonstrate acceptable method performance. If LCS recoveries are outside QC criteria, then corrective action is required. After corrective action is complete, sample re-analysis is required for failed parameters. For any other deviations from LCS control limits that cannot be resolved by sample re-analysis within holding times, the Project Chemist must be notified immediately. If samples are critically affected, the SRMT Project Manager may determine that re-sampling is required.

Matrix Spike Sample
MS recoveries are a measure of the performance of the method on the sample being analyzed. Field and trip blanks must not be chosen for spiking. MS recoveries outside the control limits applied to the LCS indicate matrix effects. Sample clean-up procedures may be warranted for samples with severe matrix effects. The laboratory should notify the Project Chemist of these instances to determine an appropriate corrective action.

Matrix Spike Duplicate Sample
The MSD sample is commonly prepared in conjunction with the MS sample. The MSD is prepared from a separate portion of the sample and processed with the same additions as the MS. The MSD is prepared for methods that do not typically show concentrations of target analytes above MDLs, such as organic methods.

Duplicate Sample
The duplicate is prepared for methods that typically show concentrations of target analytes above MDLs, such as metals and wet chemistry methods. The RPDs between recoveries in the original and duplicate measure the precision of the analytical method on the actual project samples. For this project, QC criteria for RPDs are 15 % for biota. If all other QC criteria are met, RPD results outside control limits indicate potential matrix effects. The laboratory should investigate significant deviations in the RPD results by observing the sample to determine any visual heterogeneity or reviewing sample chromatograms for matrix interference. If visual observation does not indicate a potential problem, the sample may be reanalyzed. Potential matrix effects are reported in the case narrative.

Section 12.0 INSTRUMENT/EQUIPMENT TESTING INSPECTION AND MAINTENANCE REQUIREMENTS

All instruments and equipment are inspected prior to each sampling day to ensure that all equipment is in working order and safe to use. Use of a handheld Magellan eXplorist 610 GPS unit, Radio/Cell Phone, and waterproof/shockproof Olympus Stylus 1030 SW digital camera will be needed in the field. It is important to check to make sure all units are charged prior to leaving the office, and backup batteries are packed. Prior to departure, the field crew is responsible for making sure that equipment in the Field Supplies Checklist (Appendix A) is inspected and in working condition. SRMT Environment Division has spare handheld GPS and digital cameras in the event the equipment fails.

The radio/cell phone is stored and maintained by the entrance to the SRMT Environment Office plugged in and charged for use. The field leader is responsible for making sure the radio is charged and operable. If the designated radio to this project is not operable, there are backup radios that may be used if needed located in the same place.

All laboratory instruments and equipment used for sample analysis must be serviced and maintained only by qualified personnel. Laboratory instrument maintenance procedures will be evaluated to verify that there will be no impacts on analysis of project samples due to instrument malfunction. For example, the laboratory must have duplicate instrumentation and/or major laboratory instruments (e.g., gas chromatograph/mass spectrometer) maintained under service agreements with the manufacturer that require rapid respond by manufacturer-approved service agents.

Section 13.0 INSTRUMENT CALIBRATION AND FREQUENCY

All instruments and equipment used during sampling and analysis will be operated and calibrated according to the manufacturer's guidelines and recommendations, as well as criteria set forth in applicable analytical methodology references. Personnel properly trained in these procedures will perform operation and calibration of all instruments. SRMT field staff will be responsible for calibration of balances for weight measurements, and laboratories will be responsible for equipment, apparatus, calibrations, and procedures as outlined in Appendix G. Balance operations include zero the balance prior to weighing each fish, and reading the automatically calibrated result, measured to the nearest 0.01 gram. Documentation of all field maintenance and calibration information will be maintained in the task logbook. Magellan eXplorist 610 specifications, calibrations and user manual can be found on http://www.magellangps.com/Products/eXploristseries/eXplorist-610 .

Section 14.0 INSPECTION/ACCEPTANCE REQUIREMENTS FOR SUPPLIES AND CONSUMABLES

It is the responsibility of the field crew to prepare and inspect all necessary field gear and equipment prior to beginning and throughout the sampling and processing event. A Field Supplies Checklist (Appendix A) will document supplies needed for safety, adequate communication, and field observation notations. A Fish Processing Supply Checklist (Appendix B) will document supplies needed for safety, measurements, and external gross anomaly observation notations. Confirmation this system was used will require the field crew leader to sign and date each day of survey.

Section 15.0 DATA ACQUISTION REQUIREMENTS (for Non-Direct Measurements)

As per USEPA guidance on Non-Direct Measurements in EPA QA/G-5, the rationale for selected Secondary Data Usage is outlined below. Not all sources of information have been acquired. An internet search for peer reviewed scientific literature will be conducted for selection of candidate data, as well as selection of data generated for Superfund Monitoring Repository from the facilities located within the Area of Concern.

Existing data on external abnormalities will be assessed. External abnormalities are considered a "deformity" according to historic BUI delisting considerations. In addition, it is what the public sees first as a concern for "public perception" associated with Remedial Action Committees. Alcoa has been documenting external abnormalities with their annual SRS Field Study data as a line of evidence and existing trend data. Some data on external abnormalities exists for the St. Lawrence River Area of Concern (AOC) from an Annual Fish trend monitoring conducted by ALCOA on the lower Grasse River. During ALCOA's Annual fish trend monitoring, they target 54 bullhead in the lower Grasse River within the AOC, and 5 reference fish in the upper Grasse River. Field observations and notes are made regarding abnormalities and shared with SRMT. Additionally, some data has been produced for the Cornwall Area of Concern within the Canadian boundaries in a 2010 report submitted to Environment Canada titled, "Data Analysis and Fish Tumor BUI Assessment For the Lower Great Lakes and Interconnecting Waterways" (Baumann, 2010). A Ridgway et. al. 1999 publication on White Sucker (*Catostomus commersoni*) external papillomas and contaminant burden comparison for PAHs and PCBs upstream and downstream of the Moses Saunders in the St. Lawrence River will also be reviewed to assess the Fish Tumor BUI for Massena/Akwesasne AOC. The Cornwall AOC report utilizes sampling sites specific to the north shore of the St. Lawrence River, which may not be indicative of Massena industry sources, but a potential benchmark of comparison from Outside AOC sources. The Ridgway and Alcoa field efforts report on external anomaly observations only. The external abnormalities data will be compared to Great Lakes Delisting Criteria literature values developed specific to skin lesions. For example, Bauman 2002 suggested criteria of 5% liver tumors and 12% skin tumors for distinguishing a highly contaminated AOC from less contaminated Areas of Recovery for brown bullheads. The data will be presented to the Remedial Action Committee (RAC) as a line of evidence for determining the status of the BUI based on Delisting Criteria and published literature. This, however, may not be an acceptable value to the RAC for the St. Lawrence River AOC.

Secondary data sources listed above will be reviewed only if they meet the project objectives and meet the following acceptance criteria of Data Quality Indicators (DQIs):

1. Representative: Geographically (i.e. Work done Inside AOC, or Outside AOC study area boundaries for Fish Tumor Studies), and Temporal (annual or seasonal).
2. Comparability: Are literature study results and objectives applicable and comparable? (i.e. internal and/or external anomaly observations data collection, fish health metrics, and methodologies).
3. Completeness: Did literature include a valid number of samples collected for measurement similar to sample size of interest?
4. Usability and Validity. SRMT Project Manager will review report to assess data validation and quality. If data validation and QA/QC discussion is not included in report, SRMT will contact the author to inquire if work was conducted under an approved QAPP, and the intended use of the data to determine the confidence of the data.

Reporting of the Secondary Data will be included in the Fish Tumor BUI Status Report. This will include documentation of quality procedures used and whether or not the existing data met the project's

acceptance criteria. Documentation of any programmatic or legal constraints on the use of existing data and their impact on the project will be reported. This may include data that is not available for public release or lack of background information of data generation or analysis methods.

Section 16.0 DATA MANAGEMENT

It is the responsibility of the Wildlife Technician to maintain field data sheets and field notebooks, and confirm equipment checklist. Field observations will be tabulated in a Field Data Sheet (Appendix B) and brought back from the field to the office. Field Data Sheets will be stored in a 3-ring binder only for this project with the project name on the outside cover at the SRMT Environment Division, in the Wildlife Technician's Office. Photographs of field observations will be uploaded daily and saved in dated Electronic Folders with Location ID. Field notebooks will document a summary of daily field activities and any other notes in addition to the Field Data Sheet. Field data will be entered electronically into a Microsoft Excel or Access Workbook by a Wildlife Technician.

For data collected under this project, the Project Manager will review all field data for accuracy. Any field data not provided by the laboratory will be entered into a database or spreadsheet. The laboratory will provide an electronic data deliverable (EDD) for all analytical reports that is consistent with their standard spreadsheet. The SRMT Project Manager will process the EDD and review all laboratory and field data to verify the results against the hard copy and check for transcription errors. The Project Chemist will review data against the criteria in this QAPP and add any data qualifiers. Data that will appear on data tables for the report will be generated from EDD. SRMT will develop a central data source for all data handling operations, and will retain all electronic files. Field documents and records will be retained for up to 5 years after the final report is issued.

Section 17.0 ASSESSMENTS & RESPONSE ACTIONS

17.1 Assessments

Field

The Tribal Quality Assurance Officer (TQAO) throughout the project will perform audits so that deficiencies can be found and corrected. Audits are to be conducted during the project at least once at the beginning of the sampling and at least once more during the sampling by the TQAO and SRMT Project Manager (Jessica Jock) to ensure that Field Data Sheets, Field Notebooks, Sample Handling, etc. are completed and managed properly.

Should corrective actions need to be taken due to problems that are encountered; appropriate steps will be taken to correct this. For example, if a procedure is not being followed, training or retraining of a technician may be required. Should a problem be encountered that needs corrective action involving the Sampling Program outlined in this QAPP, appropriate chain of command communication as indicated in Figure 1 will commence to effectively and efficiently rectify the problem or receive permission for any such changes. The SRMT Project Manager will be responsible for communicating to the EPA Project Manager and TQAO any problems encountered, and responsible for communicating or distributing any

corrective actions needed to the field crew. All corrective actions will be documented and explained in the field notebook and semi-reports as necessary.

Laboratory
The laboratory must implement a comprehensive program of internal audits to verify compliance of their systems with SOPs and QA manuals. NYSDOH must certify the laboratory and will perform external systems audits at an approximate frequency of once a year. External audits include reviews of analytical capabilities and procedures, COC procedures, documentation, QA/QC, and laboratory organization. No laboratory audits are planned for this project

17.2 Corrective Actions
Corrective actions will be implemented as needed. In conjunction with the QA Officer and Laboratory QA Coordinator, the Project Manager is responsible for initiating corrective action and implementing it in the field and office, and the laboratory project manager is responsible for implementing it in the laboratory. It is their combined responsibility to see that all sampling and analytical procedures are followed as specified and that the data generated meet the prescribed acceptance criteria. Specific corrective actions necessary will be clearly documented in the logbooks or analytical reports.

Field Situations
The need for corrective action in the field may be determined by technical assessments or by more direct means such as equipment malfunction. Once a problem has been identified, it may be addressed immediately or an audit report may serve as notification to project management staff that corrective action is necessary.

Immediate corrective actions taken in the field will be documented in the project logbook. Corrective actions may include, but are not limited to:

- Correcting equipment decontamination or sample handling procedures if field blanks indicated contamination;
- Recalibrating field instruments and checking battery charge;
- Training field laboratory personnel in correct sample handling or collection procedures; and
- Accepting data with an acknowledged level of uncertainty.

After a corrective action has been implemented, its effectiveness will be verified. If the action does not resolve the problem, appropriate personnel will be assigned to investigate and effectively remediate the problem.

Laboratory Situations
Out-of-control QC data, laboratory audits, or outside data review may determine the need for corrective action in the laboratory. Corrective actions may include, but are not limited to:

- Reanalyzing samples, if holding times permit;
- Correcting laboratory procedures;
- Recalibrating instruments using freshly prepared standards;
- Replacing solvents or other reagents that give unacceptable blank values;
- Training additional laboratory personnel in correct sample preparation and analysis procedures; and
- Accepting data with an acknowledged level of uncertainty.

The laboratory corrective actions must be defined in analytical SOPs. Any deviations from approved corrective actions must be documented and approved by the Project Chemist.

Section 18.0 REPORTS TO MANAGEMENT

Final Reporting of Fish Tumor Surveys will be included in a BUI Status Report on Fish Tumors or Other Deformities (scheduled to be provided to EPA and RAC Coordinator post-field study conclusions, predicted for 2013). Data summary reports will be completed by the Project Chemist and Fish Histopathologist and provided to the SRMT Project Manager for inclusion as appendices of the BUI Status Report on Fish Tumors or Other Deformities. Summary of Annual Fish Tumor Survey results (2011, 2012) will be reported accordingly in Semi-Annual (October) reporting requirements to EPA Project Officer. All reporting will be shared with the NYSDEC RAC Coordinator.

Upon completion of a project sampling effort, analytical and QC data will be included in a comprehensive technical report that summarizes field activities and provides a data evaluation. A discussion of the validity of results in the context of QA/QC procedures will be made and a data summary report will be provided.

Serious data problems will be reported immediately to SRMT personnel. Time and type of corrective action (if needed) will depend on the severity of the problem and relative overall project importance. Corrective actions may include altering procedures in the field or modifying laboratory protocol.

SRMT will implement peer review for all project deliverables including work plans, QAPPs, draft and final reports, and technical memoranda with the Massena RAC Coordinator (Steve Litwhiler) and other NYSDEC staff. The peer review process provides for a critical evaluation of the deliverable by an individual or team to determine if the deliverable will meet established criteria, quality objectives, technical standards, and contractual obligations. The Project Manager will assign peer reviewers when the publications schedule is established. The publications staff will be responsible for ensuring all peer reviewers participate in the review process and approve all final deliverables. For technical memoranda and other project documents, the Project Manager will be responsible for obtaining principal review and approval.

Section 19.0 DATA REVIEW, VALIDATION, AND VERIFICATION REQUIREMENTS

All data generated will be reviewed by comparing accuracy and precision results listed Appendices E and G.

- Field data and fish pathology results will be reviewed by internal experts for consistency with literature values. Following the pathology evaluation, the pathologist will compare prevalence results with those of previous studies as reported in the scientific literature. Differences in results may be explainable based upon sample dynamics, and/or the types of differences and frequencies encountered. If the results are substantially different, a histologic slide peer review may be recommended to resolve inconsistencies. Peer review is not currently a component of this investigation.

- All histopathology data are subject to internal QC verification and QA audit. This includes 10% of slides analyzed for diagnosis to be validated by another pathologist. When different diagnoses

occur, both pathologists should confer until they arrive at a common diagnosis. If disagreements occur for more than 25% of the samples checked, the original pathologist (Principal Investigator) should reevaluate all samples after consultation with the other pathologist. All diagnosis, discrepancies, and verification will be noted in the narrative report by the Project Histopathologist, Dr. Paul R. Bowser (Cornell University, Professor of Aquatic Animal Medicine).

- Analytical reporting limits and target compounds and QC summary data for surrogates, method blanks, LCS, and MS/MSD samples will be compared to limits listed in Appendix G.

- Calibration summary data will be checked by the laboratory to verify that all positive results for target compounds were generated under an acceptable calibration as defined by the analytical method. Any deviations will be noted in the case narrative and reviewed by the Project Chemist, Ann Casey (NEA-PACE).

- Field data such as sample identifications and sample dates will be checked against the laboratory report.

- Raw data files from the field and laboratory will be reviewed if there is a significant problem noted with the summary information.

Section 20.0 VALIDATION AND VERIFICATION METHODS

The entire project team is responsible for ongoing assessment of the technical work performed by the team, identification of nonconformance with the project objectives, and initiation, implementation and documentation of corrective action. Independent performance and systems audits are technical assessments that are a possible part of the QA/QC program. The following describes types of audits conducted, frequency of these audits, and personnel responsible for conducting audits.

20.1 Laboratory

The laboratory must implement a comprehensive program of internal audits to verify compliance of their systems with SOPs and QA manuals.

NYSDOH must certify the laboratory and will perform external systems audits at an approximate frequency of once a year. External audits include reviews of analytical capabilities and procedures, COC procedures, documentation, QA/QC, and laboratory organization.

No laboratory audits are planned for this project.

20.2 Field

All data generated during the field activities will be general information, sample identification numbers, date, time, sample matrix, weights, and lengths, etc. and field data will be noted in pen or sharpie in a field notebook and field data sheets.

20.3 Audits

To be performed by the SRMT Project Manager on the field employees at least once prior to field tracking event. Field employees will have thoroughly reviewed the QAPP and are familiar with the basic procedures before Fish Tumor Survey efforts.

20.4 Corrective Actions

All personnel involved with the study shall follow the QA/QC Sampling Plan to avoid errors that may occur in the field. If a sample is not attainable from a designated sampling location, then another sampling area will be chosen as described in Section 7.0, based on home range and AOC boundaries. See Section 17.2 for more specifics on Corrective Actions.

20.5 Reports

The final report will include all data that has been attained throughout the study in text, tabular and graphical form in Microsoft Programming (i.e., Excel, Word, and/or Access) and GIS mapping tools. The final report will be completed when all data has been reviewed and submitted to the St. Regis Mohawk Tribe Environment Division where the final review and report compilation will take place.

Section 21.0 RECONCILIATION WITH DATA QUALITY OBJECTIVES

Project DQOs will be evaluated and discussed in October Semi-Annual Reporting Periods with the USEPA Project Officer. Power of statistics will be demonstrated, and reported in the *BUI Status Report on Fish Tumors and Other Deformities.*

Any deviations from analytical performance criteria or quality objectives for the project will be documented in the data summary report provided to the data users for the project. The QA Officer or Project Chemist will work with the final users of the data in performing data quality assessments. The data quality assessment may include some or all of the following steps:

- Data that are determined to be incomplete or not usable for the project will be discussed with the project team. If critical data points are involved which impact the ability to complete project objectives, data users will report immediately to the Project Manager. The Project Manager will discuss resolution of the issue with technical staff and implement necessary corrective actions (for example re-sampling);
- Data that are non-detect but have elevated reporting limits due to blank contamination or matrix interference will be compared to background values. If incomplete data as described above; and
- Data that are qualified as estimated will be used for all project decision making.

Data assessors comparing to results to background will have to account for the higher level of uncertainty in their statistical analysis. Part of the assessment process involves comparing analytical results for bullheads background concentrations to determine if the contamination present is interrelated (i.e., above background levels). If the total PCB and PAH concentrations are above background, then the concentrations will be compared to appropriate literature values and other related scientific studies to determine the potential significance of the concentration levels.

The usability of all data types for making comparisons between the site and reference area will be ensured through careful attention to the following factors:

- Selection of a suitable reference area. The chosen reference area will be highly ecologically similar to the site. The proposal for sampling inside vs. outside the AOC in each of the tributaries is the mechanism to ensure ecological similarity. It is assumed that the Grasse River is not similar to the Raquette River, and the Raquette is not similar to the St. Regis. In addition, all sampling will be conducted in the shallows via hoop net with a river section similar to a run or pool, and during spring pre-spawn. The reference site will support, or be capable of supporting, the same fish species as the site so that

differences between the site and reference area due to chemical contamination will not be confounded with habitat effects. No sediment data is needed to obtain the data needs of this study. There is a significant amount of historical data for this Area of Concern, as well as for reference locations. The reviewer can obtain this information on-line at NOAA:
http://response.restoration.noaa.gov/type_subtopic_entry.php?RECORD_KEY%28entry_subtopic_type%29=entry_id,subtopic_id,type_id&entry_id(entry_subtopic_type)=386&subtopic_id(entry_subtopic_type)=36&type_id(entry_subtopic_type)=3

- Scientifically sound, acceptable methods will be used for all data collection activities so that differences between the site and reference area, if identified, can be considered real, not artifacts of sample collection.

- Competent experienced biologists will be involved in all aspects of field work to ensure that only high-quality data are collected.

- Rigorous procedures and checks will be in place to ensure that all project data are accurately recorded and incorporated into the project database.

Section 22.0 LITERATURE REFERENCED

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Figure 3-1. St. Lawrence River Inside the AOC (Site #1)

Figure 3-2. St. Lawrence River Outside the AOC (Site #2)

Figure 3-3. Grasse River Inside the AOC (Site #3)

Figure 3-4. Grasse River Outside the AOC (Site #4)

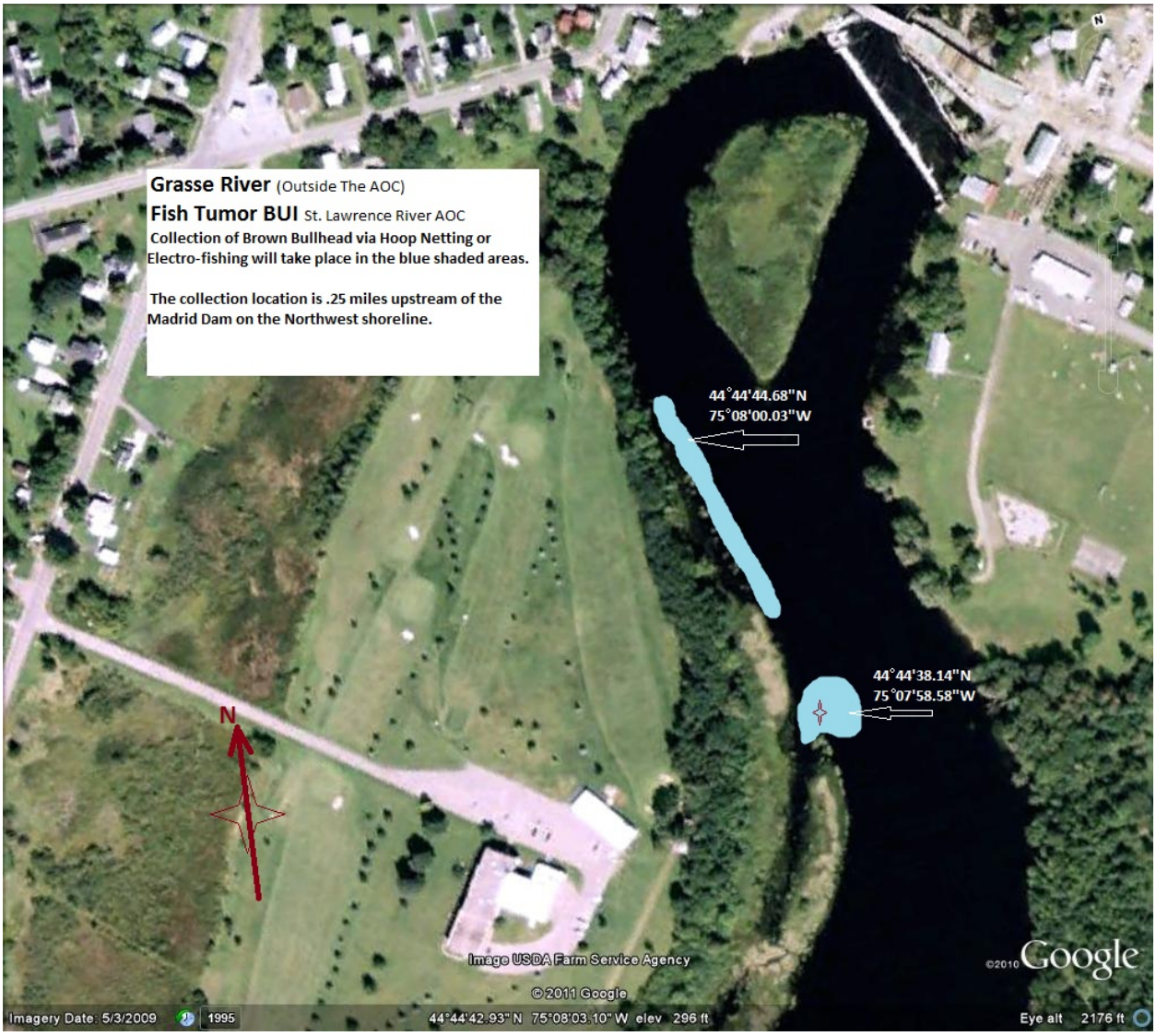


Figure 3-5. Raquette River Inside the AOC (Site #5)

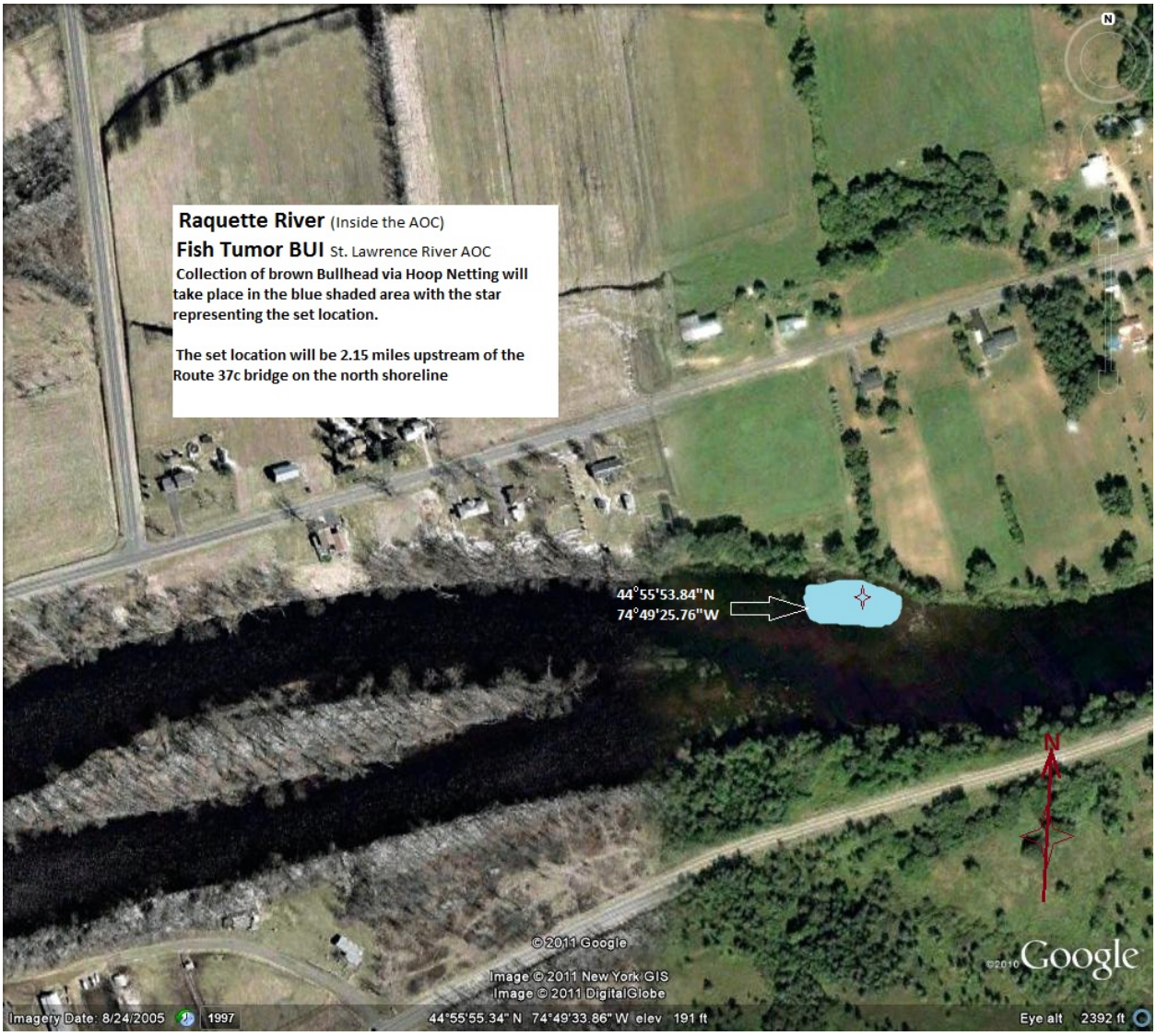
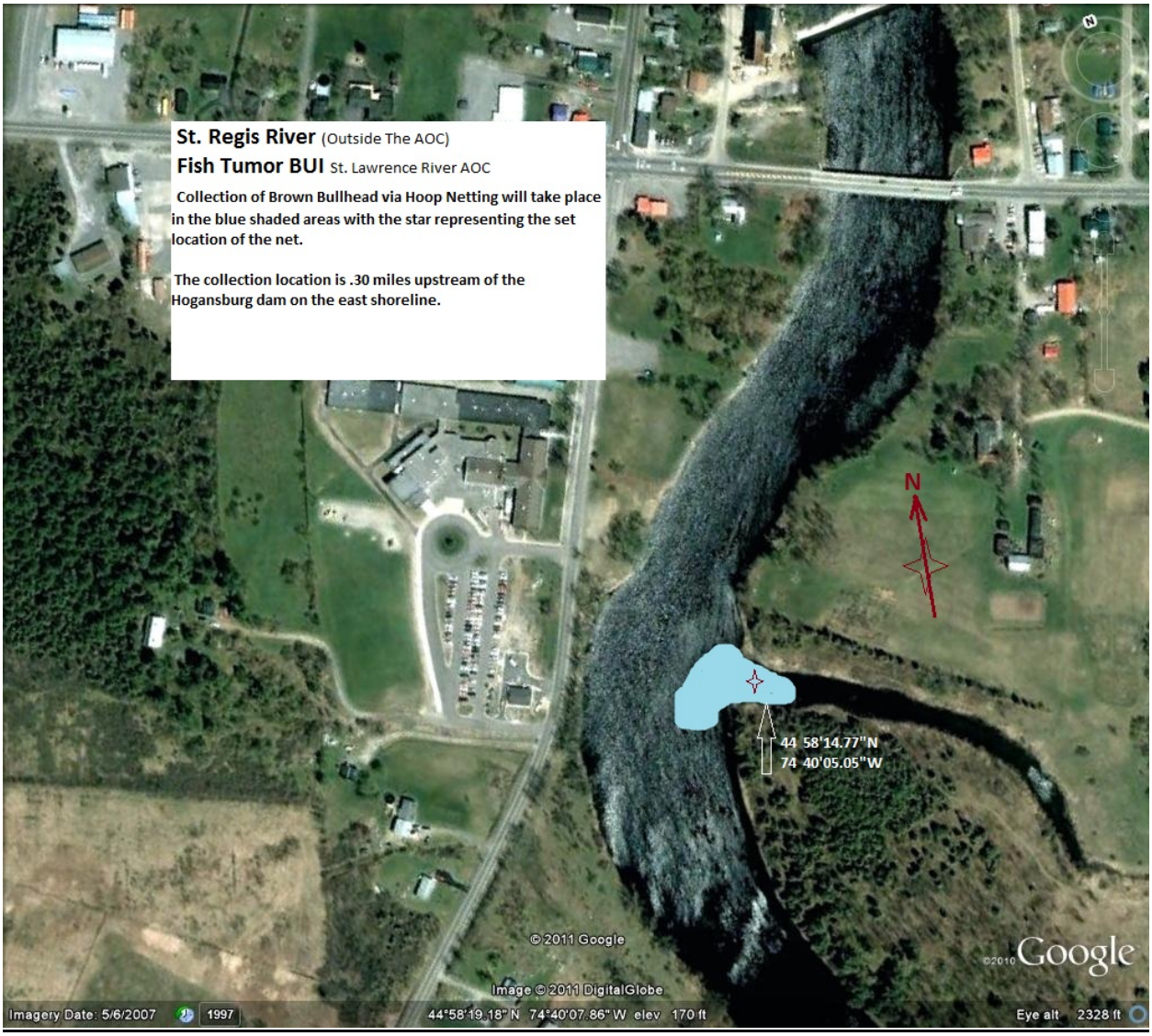


Figure 3-6. Raquette River Outside the AOC (Site #6)

Figure 3-7. St. Regis River Inside the AOC (Site #7)

Figure 3-8. St. Regis River Outside the AOC (Site #8)



Appendix A. Field Collection Supplies Checklist

Field Collection Supplies Checklist:

- ___ Portable Radio and/or Cell Phone
- ___ First Aid Kit
- ___ PFD personal floatation device
- ___ Map of Area
- ___ Field DATA Sheets
- ___ All Weather Field Book
- ___ "Rite in the Rain" pen or Sharpie
- ___ Labels for fish specimens
- ___ Fish Scale Envelopes, "Rite in the Rain"
- ___ ID Books
- ___ Digital camera (PENTAX W90)
- ___ Handheld GPS
- ___ Flashlight
- ___ Extra Batteries
- ___ Dry Sack with spare (socks, gloves, map, batteries)
- ___ Rope
- ___ Gerber Multi-plier Tool
- ___ Rain Gear
- ___ 6 inch & 18 inch Ruler
- ___ Measuring Board
- ___ Weighing Scales
- ___ Buckets, Tubs, and Live Wells
- ___ Plastic Bags; Plastic Wrap; heavy Duty Aluminum Foil; Paper towels
- ___ Polyethylene disposable, non-powdered gloves

- ___ Ice Chests & Ice
- ___ Neoprene Coated Utility Gloves
- ___ Armored Water Thermometer
- ___ DEC Collector's License

Boat Supplies Checklist

- ___ Fire Extinguisher
- ___ Dip Nets
- ___ Waders
- ___ Safety Equipment Checklist
- ___ Eye Protection
- ___ Sun Protection
- ___ Lip Grip Scales

Hoop Net Supplies Checklist

- ___ Seine Twine for repairs
- ___ Polypropylene Rope
- ___ Snap Hooks
- ___ Extra Floats
- ___ Weights
- ___ Rebar Stakes
- ___ Lip Grip Scales
- ___ Photo Identification Markers
- ___ Waders
- ___ Identification Buoy

Field Crew Leader Signature Date

Appendix B. Fish Processing Supply Checklist

Fish Processing Supply Checklist

- ____ First Aid Kit
- ____ Chain of Custody forms
- ____ All Weather Field Book
- ____ Laboratory Notes Book
- ____ Plastic Bags; Plastic Wrap; Heavy Duty Aluminum Foil; Paper Towels
- ____ Fish Scale Envelopes “Rite in the Rain”
- ____ Waterproof Labels
- ____ “Rite in the Rain” Pens or Sharpies
- ____ Index Cards
- ____ Digital Camera (PENTAX w90)
- ____ Reference Manuals for brown bullhead (2)
- ____ Internal Anatomy Diagram
- ____ Polyethylene disposable, non-powdered gloves
- ____ Chem. Resistant Lab Apron
- ____ Sterile Specimen Containers, 250ml
- ____ Seal Storage Bags
- ____ Anti-fog Goggles
- ____ Extra-large 2.5x Viewer
- ____ Open Digital Scale
- ____ Fish Measuring Board
- ____ Anatomy Dissecting Set
- ____ Extra Scalpel Handles & Blades
- ____ Satterlee Bone Saw, 7 ½ inch

- ____ 6-Piece Fillet Set
- ____ 10% Buffered Formalin
- ____ Hexane Rinse
- ____ De-ionized Water(H20)
- ____ MSDS for all Chemicals
- ____ Waste Buckets for Fish & Chemicals
- ____ Garbage Bags
- ____ Duct Tape

Crew Leader
Signature_____Date_____

Appendix C. Field Manual for Assessing Internal and External Anomalies in Brown Bullhead (*Ameiurus nebulosus*)(Rafferty and Grazio, 2006) and Field Data Sheets

http://seagrant.psu.edu/publications/technicaldocs/BullheadFieldManual.pdf

FISH HEALTH DATA SHEET

Collection Date:	Process Date:	Time: AM / PM	Field Observers:	Sample ID:
___/___/____	___/___/____	_____ : _____	_____	

Water Condition: _____ **Water Temp:** _____ **Air Temp:** _____

Location: _____	Latitude	Longitude
River: *St.Lowrence, Grasse, Raquette, St. Regis, Salmon*	_____°N	_____°W

Capture Gear: Trapnet___ Hoopnet___ Electro fishing___ Angling___ Other___, _____

Histopathology _____	Species: *Brown Bullhead___ Yellow Bullhead___ White Sucker___*

Sex: M F	Total Length: (mm)_____ Total Weight: (grams)_____	Age: _____ *Spines or Otoliths*

Fish Health Information: Clean? Yes_____ No_____ (Note Physical Condition Below)

Pictures Taken of Sample Fish: *Yes____, No____* File # of Pictures: _____

_____

Signature (recorder)_____ **Date**_____

Signature (Reviewer)_____ **Date**_____

Severity Score *0-No Visible maladies 1-Mild Condition 2-Moderate 3-Severe*

Barbels	0 1 2 3	Barbels-Notes
RAISED Skin Lesions	0 1 2 3	Skin Lesion-Notes
RAISED Mouth Lesions	0 1 2 3	Mouth Lesion-Notes
Pigmentation(Yellow)	0 1 2 3	Yellow Pigmentation-Notes
Pigmentation(Black)	0 1 2 3	Black Pigmentation-Notes
Fin Erosion	0 1 2 3	Fin Erosion-Notes
Ulcers	0 1 2 3	Ulcer-Notes
Scars/Wounds	0 1 2 3	Scars/Wounds-Notes
Eyes	0 1 2 3	Eyes-notes
Tissue Chemistry	Yes No	Whole Fish____ Liver Sample____ Fillet Sample____

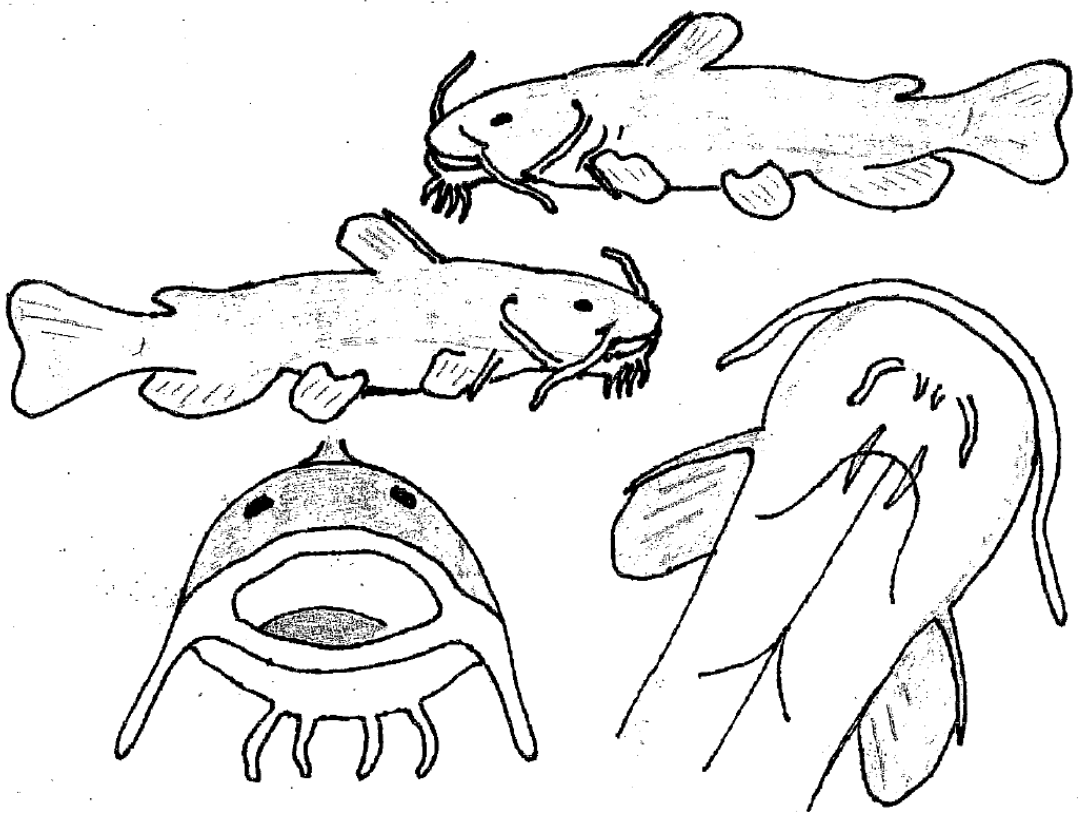
INTERNAL EXAMINATION: *(check all that apply)* FISH ID# _____

LIVER: ☐OTHER *specify:* _____ Weight _____(0.1g) *with gallbladder intact*
☐dark to light red
☐tan (coffee with cream) _____ _____ (Cryovial 1)
☐general discoloration _____ _____ (Cryovial 2)
☐focal discoloration _____
☐nodules _____ # in fixative _____ # of Photos _____

BILE: Color: Fullness: ☐OTHER *specify:* _____
☐yellow ☐empty
☐light-grass green ☐partly full
☐dark green to blue-green ☐full
_____ # of Photos _____

SPLEEN: ☐OTHER *specify:* _____ Weight _____(0.002g)
☐red to black
☐granular _____ # in fixative _____
☐nodular _____
☐enlarged _____ # of Photos _____

GONADS: ☐OTHER *specify:* _____ ☐ripe Weight _____(0.1g)
☐male ☐spent
☐female _____ ☐intermediate
☐intermediate _____
☐juvenile # in fixative _____ # of Photos _____



Notes on severity rankings of gross external anomalies in Field Manual for Assessing Internal and External Anomalies in Brown Bullhead (PA Sea Grant and PADEP, December 2006)[1]

Category	Severity Ranking	Photograph Depiction
Barbels	1 mild	1 shortened/missing/deformed barbel
	2 moderate	2 shortened/missing/deformed barbels
	3 severe	* >2 shortened/missing/deformed barbels
Raised Skin Lesions	1 mild	1 lesion
	2 moderate	2 lesions
	3 severe	many
Raised Mouth Lesions	1 mild	1 small area
	2 moderate	1 large area
	3 severe	2 large areas
Pigmentation (Yellow or Black)	1 mild	1 spot
	2 moderate	2 to 4 spots
	3 severe	extensive coverage
Fin Erosion	1 mild	* 1 small area on fin
	2 moderate	* 1 large area on fin
	3 severe	2 fins; extensive coverage
Ulcers	1 mild	1 small ulcer
	2 moderate	1 large ulcer
	3 severe	2 ulcers
Scars/Wounds	1 mild	* 1 healed area
	2 moderate	1 small area
	3 severe	1 large area
Eyes	1 mild	1 discolored eye
	2 moderate	1 cloudy or missing eye
	3 severe	* 2 missing eyes

Notes:
1. The notes are based on photograph depictions contained in the manual
* = No photo present in manual; severity assumed based on other rankings

Appendix D. Illustrated Field Guide for Assessing External and Internal Anomalies in Fish (USGS/ERD/ITR – 2002-0007) (pdf attachment)

http://www.cerc.usgs.gov/pubs/center/pdfDocs/ITR_2002_0007.pdf

Appendix E. Manual for the Microscopic Diagnosis of Proliferative Liver and Skin Lesions in the Brown Bullhead (*Ameiurus nebulosus*) (Blazer et. al 2007)

http://seagrant.psu.edu/publications/technicaldocs/HistoFieldManual.pdf

Appendix F. Laboratory Experience (pdfs attached)

Includes:

1. **NEA, Inc, A Division of PACE Analytical Services ELAP Certifications (electronic pdf.)**
2. **Fish Pathologist Curriculum Vitae (C.V.)**

Appendix G. Chemical Laboratory SOPs (pdfs attachment)

1. **Extraction and Cleanup of Fish and Biota Materials (NE017_09)**
2. **The Extraction of Fish and Biota Material (NE158_05_01): Reference Methods: EPA Method 3500A & 3600A**
3. **Analysis of PAHs in Solid Sample Extracts by GC-MS (NE238_05): Reference Methods: SW-846 Method 8270D-SIM**
4. **Tissue and Preparation & Homogenization For Biota and Plant Matrices (NEA132-07). Reference Methods: US EPA SW-846 Test Methods for Evaluating Solid Waste**
5. **Quality Assurance Manual. Quality Assurance/Quality Control Policies and Procedures for Pace Analytical Services – NEA, A Division of Pace Analytical. Approved November 15, 2010.**

Appendix H. Laboratory Chain of Custody